

# pK<sub>a</sub> measurements for the SAMPL6 prediction challenge for a set of kinase inhibitor-like fragments

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## SUPPLEMENTARY INFORMATION

**Table SI 1.** Procurement details of SAMPL6 pK<sub>a</sub> challenge compounds

SAMPL6 Molecule ID	Group	Supplier	LOT	CAT	Supplier reported purity	CAS	eMolecules ID	canonical isomeric SMILES	Experimental Molecule ID
SM01	fragment-like	AchemBlock	11549	10222	95%	521937-07-05	6679830	<chem>c1cc2c(cc1O)c3c(o2)C(=O)NCCC3</chem>	M01
SM02	fragment-like	ChemDiv	CM02432403	3232-0333			1327907	<chem>c1ccc2c(c1)c(ncn2)Nc3cccc(c3)C(F)(F)F</chem>	M02
SM03	fragment-like	ChemDiv		Z27474679			1228629	<chem>c1ccc(cc1)Cc2nnc(s2)NC(=O)c3cccs3</chem>	M03
SM04	fragment-like	ChemDiv		Z126957826			30719859	<chem>c1ccc2c(c1)c(ncn2)NCc3ccc(cc3)Cl</chem>	M04
SM05	fragment-like	ChemDiv		Z119335440			18908671	<chem>c1ccc(c(c1)NC(=O)c2ccc(o2)Cl)N3CCCCC3</chem>	M05
SM06	fragment-like	ChemDiv		Z28487401			18893169	<chem>c1cc2cccn2c(c1)NC(=O)c3cc(cnc3)Br</chem>	M06
SM07	fragment-like	Enamine	2017-0168841	Z57161635	95%	100818-54-0	1327878	<chem>c1ccc(cc1)CNC2c3ccccc3ncn2</chem>	M07
SM08	fragment-like	Enamine	2017-0168838	Z57157353	95%	65418-08-8	1367649	<chem>Cc1ccc2c(c1)c(c(c(=O)[nH]2)CC(=O)O)c3ccccc3</chem>	M08
SM09	fragment-like	Enamine	2017-0168839	Z220564816	95%		1865544	<chem>COc1ccc(c(c1)Nc2c3ccccc3ncn2)Cl</chem>	M09
SM10	fragment-like	Enamine	2017-0168843	Z69130143	95%	35056-22-5	23354217	<chem>c1ccc(cc1)C(=O)NCC(=O)Nc2nc3ccccc3s2</chem>	M10
SM11	fragment-like	Maybridge	142989	RJC00689SC	90%	5334-30-5	719540	<chem>c1ccc(cc1)n2c3c(cn2)c(ncn3)N</chem>	M11
SM12	fragment-like	Maybridge	265423	DP00818SC			1859493	<chem>c1ccc2c(c1)c(ncn2)Nc3cccc(c3)Cl.Cl</chem>	M12
SM13	fragment-like	Maybridge	248841	GK03474SC			5828805	<chem>Cc1cccc(c1)Nc2c3cc(c(cc3ncn2)OC)OC</chem>	M13
SM14	fragment-like	Enamine		Z57290870			31653344	<chem>c1ccc(cc1)n2cnc3c2ccc(c3)N</chem>	M15
SM15	fragment-like	Enamine		Z1318268952			37095168	<chem>c1ccc2c(c1)ncn2c3ccc(cc3)O</chem>	M16
SM16	fragment-like	VitaScreen		STK098832			1284691	<chem>c1cc(c(c(c1)Cl)C(=O)Nc2ccncc2)Cl</chem>	M18
SM17	fragment-like	VitaScreen		STK032731			1444229	<chem>c1ccc(cc1)CSc2nnc(o2)c3ccncc3</chem>	M19
SM18	drug-like	Enamine		Z278071350			18897105	<chem>c1ccc2c(c1)c(=O)[nH]c(n2)CCC(=O)Nc3ncc(s3)Cc4ccc(c(c4)F)F</chem>	D01
SM19	drug-like	Enamine		Z30206127			3365457	<chem>CCOc1ccc2c(c1)sc(n2)NC(=O)Cc3ccc(c(c3)Cl)Cl</chem>	D02
SM20	drug-like	VitaScreen		STL282831			46568819	<chem>c1cc(cc(c1)OCc2ccc(cc2Cl)Cl)/C=C/3\C(=O)NC(=O)S3</chem>	D05
SM21	drug-like	VitaScreen		STL368658			1574612	<chem>c1cc(cc(c1)Br)Nc2c(cnc(n2)Nc3cccc(c3)Br)F</chem>	D06
SM22	drug-like	VitaScreen		STK070581			536848	<chem>c1cc2c(cc(c(c2nc1)O))I</chem>	D07
SM23	drug-like	VitaScreen		STK097966			4375254	<chem>CCOC(=O)c1ccc(cc1)Nc2cc(nc(n2)Nc3ccc(cc3)C(=O)OCC)C</chem>	D08
SM24	drug-like	VitaScreen		STK090644			1415746	<chem>COc1ccc(cc1)c2c3c(ncnc3oc2c4ccc(cc4)OC)NCCO</chem>	D09

**Table SI 2.** Calculated properties and descriptors of compounds of 28 compounds selected and procured for pKa challenge. pKa measurement experiments were successful 24 molecules labeled SM01-SM24.

SAMPL6 Molecule ID	Experimental Molecule ID	group	Epik pK <sub>a</sub> s in [3,11] range	OpenEye XlogP	Molecular Weight (g/mol)	eMolecules reported availability (mg)	Number of rotatable bonds	Number of UV-chr. units	eMolecules ID	canonical isomeric SMILES
SM01	M01	fragment-like	[9.119]	3.27	289.26	184	0	27	6679830	<chem>c1cc2c(cc1O)c3c(o2)C(=O)NCCC3</chem>
SM02	M02	fragment-like	[4.05]	3.61	301.39	101	3	36	1327907	<chem>c1ccc2c(c1)c(ncn2)Nc3cccc(c3)C(F)(F)F</chem>
SM03	M03	fragment-like	[7.12]	3.52	269.73	379	5	14	1228629	<chem>c1ccc(cc1)Cc2nnc(s2)NC(=O)c3cccs3</chem>
SM04	M04	fragment-like	[5.564]	3.79	304.77	415.5	3	36	30719859	<chem>c1ccc2c(c1)c(ncn2)NCC3ccc(cc3)Cl</chem>
SM05	M05	fragment-like	[5.346]	2.92	328.16	424.3	4	18	18908671	<chem>c1ccc(c(c1)NC(=O)c2ccc(o2)Cl)N3CCCCC3</chem>
SM06	M06	fragment-like	[4.001, 10.328]	2.91	235.28	406	3	37	18893169	<chem>c1cc2ccnc2c(c1)NC(=O)c3cc(cnc3)Br</chem>
SM07	M07	fragment-like	[5.564]	2.80	293.32	208.7	3	36	1327878	<chem>c1ccc(cc1)CNc2c3cccc3ncn2</chem>
SM08	M08	fragment-like	[4.109]	3.14	287.74	232.1	3	59	1367649	<chem>Cc1ccc2c(c1)c(c(c(=O)[nH]2)CC(=O)O)c3ccccc3</chem>
SM09	M09	fragment-like	[4.05]	2.72	311.36	119.7	3	36	1865544	<chem>COc1cccc(c1)Nc2c3cccc3ncn2.Cl</chem>
SM10	M10	fragment-like	[8.672]	1.50	211.22	149.1	6	28	23354217	<chem>c1ccc(cc1)C(=O)NCC(=O)Nc2nc3cccc3s2</chem>
SM11	M11	fragment-like	[3.869]	3.90	292.16	3430	1	31	719540	<chem>c1ccc(cc1)n2c3c(cn2)c(ncn3)N</chem>
SM12	M12	fragment-like	[4.05]	2.60	295.34	7366	2	36	1859493	<chem>c1ccc2c(c1)c(ncn2)Nc3cccc(c3)Cl.Cl</chem>
SM13	M13	fragment-like	[4.267]	2.91	235.28	1864	4	36	5828805	<chem>Cc1cccc(c1)Nc2c3cc(c(cc3ncn2)OC)OC</chem>
-	M14	fragment-like	[5.564]	2.33	209.25	208.7	3	36	1327878	<chem>c1ccc(cc1)CNc2c3cccc3ncn2</chem>
SM14	M15	fragment-like	[6.348]	2.22	210.23	50213	1	40	31653344	<chem>c1ccc(cc1)n2cnc3c2ccc(c3)N</chem>
SM15	M16	fragment-like	[5.82, 8.709]	4.23	263.33	21650.2	1	40	37095168	<chem>c1ccc2c(c1)ncn2c3ccc(cc3)O</chem>
-	M17	fragment-like	[3.158]	2.93	267.11	283.7	2	24	45809595	<chem>CC(C)c1ccc(cc1)/C=C\2/c3ccccc3NC2=O</chem>
SM16	M18	fragment-like	[4.714, 9.847]	3.31	269.32	385	3	20	1284691	<chem>c1cc(c(c(c1)Cl)C(=O)Nc2ccncc2)Cl</chem>
SM17	M19	fragment-like	[4.902]	3.34	426.44	170	4	30	1444229	<chem>c1ccc(cc1)CSc2nnc(o2)c3cncc3</chem>
SM18	D01	drug-like	[9.381, 10.773]	5.17	381.28	247.7	7	37	18897105	<chem>c1ccc2c(c1)c(=O)[nH]c(n2)CCC(=O)Nc3ncc(s3)Cc4ccc(c(c4)F)F</chem>
SM19	D02	drug-like	[9.167]	5.78	403.31	489.9	6	28	3365457	<chem>CCOc1ccc2c(c1)sc(n2)NC(=O)Cc3ccc(c(c3)Cl)Cl</chem>
-	D03	drug-like	[4.113]	4.72	401.48	324.5	6	35	10794751	<chem>CC(C)(C)c1cc(n(n1)c2cccc2)NC(=O)Nc3cccc(c3)Cl</chem>
-	D04	drug-like	[3.199]	5.24	380.25	636.9	5	34	3064762	<chem>c1ccc(cc1)C(=O)Nc2ccc(cc2)Oc3c4c5c(sc4ncn3)CCCC5</chem>
SM20	D05	drug-like	[8.05]	4.14	438.09	154	4	24	46568819	<chem>c1cc(cc(c1)OCc2ccc(cc2)Cl)/C=C/3\C(=O)NC(=O)S3</chem>
SM21	D06	drug-like	[3.892]	3.37	396.95	222	4	28	1574612	<chem>c1cc(cc(c1)Br)Nc2c(cnc(n2)Nc3cccc(c3)Br)F</chem>
SM22	D07	drug-like	[3.511, 6.794]	2.94	420.46	239	0	29	536848	<chem>c1cc2c(cc(c(c2nc1)O))I</chem>
SM23	D08	drug-like	[6.336]	2.79	391.42	319	10	28	4375254	<chem>CCOC(=O)c1ccc(cc1)Nc2cc(nc(n2)Nc3ccc(cc3)C(=O)OCC)C</chem>
SM24	D09	drug-like	[4.829]	3.27	289.26	398	7	71	1415746	<chem>COc1ccc(cc1)c2c3c(ncnc3oc2c4ccc(cc4)OC)NCCO</chem>

**Table SI 3.** pK<sub>a</sub> results of replicate UV-metric pK<sub>a</sub> measurements. "UV-metric pK<sub>a</sub>" assay indicates spectrophotometric pK<sub>a</sub> measurements done with Sirius T3 in ISA water. "UV-metric pK<sub>a</sub> with cosolvent" assay refers to pK<sub>a</sub> determination by Yasuda-Shedlovsky extrapolation from p<sub>s</sub>K<sub>a</sub> measurements in various ratios of ISA methanol:water mixtures. Triplicate measurements were performed at 25 ± 0.5°C and in the presence of approximately 150 mM KCl to adjust ionic strength.

Molecule ID	pK <sub>a1</sub>	pK <sub>a2</sub>	pK <sub>a3</sub>	Assay Type	Experiment ID	Experiment Report
SM01	9.54			UV-metric pK <sub>a</sub>	17I-15024	SM01_17I-15024_M01_UV-metric pKa_report.pdf
SM01	9.53			UV-metric pK <sub>a</sub>	17I-15025	SM01_17I-15025_M01_UV-metric pKa_report.pdf
SM01	9.53			UV-metric pK <sub>a</sub>	17I-16001	SM01_17I-16001_M01_UV-metric pKa_report.pdf
SM02	5.04			UV-metric pK <sub>a</sub> with cosolvent	17I-22022	SM02_17I-22022_M02_UV-metric psKa_report.pdf
SM02	5.04			UV-metric pK <sub>a</sub> with cosolvent	17I-22023	SM02_17I-22023_M02_UV-metric psKa_report.pdf
SM02	5.02			UV-metric pK <sub>a</sub> with cosolvent	17I-22024	SM02_17I-22024_M02_UV-metric psKa_report.pdf
SM03	7.01			UV-metric pK <sub>a</sub> with cosolvent	17I-19004	SM03_17I-19004_M03_UV-metric psKa_report.pdf
SM03	7.01			UV-metric pK <sub>a</sub> with cosolvent	17I-19005	SM03_17I-19005_M03_UV-metric psKa_report.pdf
SM03	7.03			UV-metric pK <sub>a</sub> with cosolvent	17I-19006	SM03_17I-19006_M03_UV-metric psKa_report.pdf
SM04	6.03			UV-metric pK <sub>a</sub>	17I-18018	SM04_17I-18018_M04_UV-metric pKa_report.pdf
SM04	6.02			UV-metric pK <sub>a</sub>	17I-18019	SM04_17I-18019_M04_UV-metric pKa_report.pdf
SM04	6.02			UV-metric pK <sub>a</sub>	17I-18020	SM04_17I-18020_M04_UV-metric pKa_report.pdf
SM05	4.57			UV-metric pK <sub>a</sub> with cosolvent	17I-19007	SM05_17I-19007_M05_UV-metric psKa_report.pdf
SM05	4.6			UV-metric pK <sub>a</sub> with cosolvent	17I-19008	SM05_17I-19008_M05_UV-metric psKa_report.pdf
SM05	4.6			UV-metric pK <sub>a</sub> with cosolvent	17I-19009	SM05_17I-19009_M05_UV-metric psKa_report.pdf
SM06	2.96	11.76		UV-metric pK <sub>a</sub>	17I-18021	SM06_17I-18021_M06_UV-metric pKa_report.pdf
SM06	3.11	11.74		UV-metric pK <sub>a</sub>	17I-18022	SM06_17I-18022_M06_UV-metric pKa_report.pdf
SM06	3.02	11.71		UV-metric pK <sub>a</sub>	17I-18023	SM06_17I-18023_M06_UV-metric pKa_report.pdf
SM07	6.07			UV-metric pK <sub>a</sub>	17I-16002	SM07_17I-16002_M07_UV-metric pKa_report.pdf
SM07	6.07			UV-metric pK <sub>a</sub>	17I-16004	SM07_17I-16004_M07_UV-metric pKa_report.pdf
SM07	6.09			UV-metric pK <sub>a</sub>	17I-20001	SM07_17I-20001_M07_UV-metric pKa_report.pdf
SM08	4.23			UV-metric pK <sub>a</sub>	17I-19001	SM08_17I-19001_M08_UV-metric pKa_report.pdf
SM08	4.2			UV-metric pK <sub>a</sub>	17I-19002	SM08_17I-19002_M08_UV-metric pKa_report.pdf
SM08	4.22			UV-metric pK <sub>a</sub>	17I-19003	SM08_17I-19003_M08_UV-metric pKa_report.pdf
SM09	5.37			UV-metric pK <sub>a</sub> with cosolvent	17I-16014	SM09_17I-16014_M09_UV-metric psKa_report.pdf
SM09	5.35			UV-metric pK <sub>a</sub> with cosolvent	17I-16015	SM09_17I-16015_M09_UV-metric psKa_report.pdf
SM09	5.4			UV-metric pK <sub>a</sub> with cosolvent	17I-16016	SM09_17I-16016_M09_UV-metric psKa_report.pdf
SM10	9.01			UV-metric pK <sub>a</sub> with cosolvent	17I-20020	SM10_17I-20020_M10_UV-metric psKa_report.pdf
SM10	9.02			UV-metric pK <sub>a</sub> with cosolvent	17I-20021	SM10_17I-20021_M10_UV-metric psKa_report.pdf
SM10	9.02			UV-metric pK <sub>a</sub> with cosolvent	17I-20022	SM10_17I-20022_M10_UV-metric psKa_report.pdf
SM11	3.89			UV-metric pK <sub>a</sub>	17I-16005	SM11_17I-16005_M11_UV-metric pKa_report.pdf
SM11	3.89			UV-metric pK <sub>a</sub>	17I-16006	SM11_17I-16006_M11_UV-metric pKa_report.pdf
SM11	3.89			UV-metric pK <sub>a</sub>	17I-16007	SM11_17I-16007_M11_UV-metric pKa_report.pdf
SM12	5.28			UV-metric pK <sub>a</sub>	17I-21002	SM12_17I-21002_M12_UV-metric pKa_report.pdf
SM12	5.28			UV-metric pK <sub>a</sub>	17I-21003	SM12_17I-21003_M12_UV-metric pKa_report.pdf
SM12	5.27			UV-metric pK <sub>a</sub>	17I-21004	SM12_17I-21004_M12_UV-metric pKa_report.pdf
SM13	5.79			UV-metric pK <sub>a</sub>	17I-16011	SM13_17I-16011_M13_UV-metric pKa_report.pdf
SM13	5.76			UV-metric pK <sub>a</sub>	17I-16012	SM13_17I-16012_M13_UV-metric pKa_report.pdf
SM13	5.76			UV-metric pK <sub>a</sub>	17I-16013	SM13_17I-16013_M13_UV-metric pKa_report.pdf

**Table SI 3.** Continued.

Molecule ID	pK <sub>a1</sub>	pK <sub>a2</sub>	pK <sub>a3</sub>	Assay Type	Experiment ID	Experiment Report
SM14	2.59	5.31		UV-metric pK <sub>a</sub>	17I-29002	SM14_17I-29002_M15_UV-metric pKa_report.pdf
SM14	2.57	5.29		UV-metric pK <sub>a</sub>	17I-29003	SM14_17I-29003_M15_UV-metric pKa_report.pdf
SM14	2.57	5.29		UV-metric pK <sub>a</sub>	17I-29004	SM14_17I-29004_M15_UV-metric pKa_report.pdf
SM15	4.71	8.96		UV-metric pK <sub>a</sub>	17K-10009	SM15_17K-10009_M16_UV-metric pKa_report.pdf
SM15	4.7	8.94		UV-metric pK <sub>a</sub>	17K-10010	SM15_17K-10010_M16_UV-metric pKa_report.pdf
SM15	4.69	8.92		UV-metric pK <sub>a</sub>	17K-10011	SM15_17K-10011_M16_UV-metric pKa_report.pdf
SM16	5.37	10.64		UV-metric pK <sub>a</sub>	17J-03025	SM16_17J-03025_M18_UV-metric pKa_report.pdf
SM16	5.37	10.65		UV-metric pK <sub>a</sub>	17J-03026	SM16_17J-03026_M18_UV-metric pKa_report.pdf
SM16	5.38	10.65		UV-metric pK <sub>a</sub>	17J-03027	SM16_17J-03027_M18_UV-metric pKa_report.pdf
SM17	3.15			UV-metric pK <sub>a</sub>	17J-02024	SM17_17J-02024_M19_UV-metric pKa_report.pdf
SM17	3.16			UV-metric pK <sub>a</sub>	17J-02025	SM17_17J-02025_M19_UV-metric pKa_report.pdf
SM17	3.17			UV-metric pK <sub>a</sub>	17J-02026	SM17_17J-02026_M19_UV-metric pKa_report.pdf
SM18	2.1	9.51	10.93	UV-metric pK <sub>a</sub> with cosolvent	17I-28001	SM18_17I-28001_D01_UV-metric psKa_report.pdf
SM18	2.15	9.59	11.03	UV-metric pK <sub>a</sub> with cosolvent	17I-28002	SM18_17I-28002_D01_UV-metric psKa_report.pdf
SM18	2.19	9.63	11.11	UV-metric pK <sub>a</sub> with cosolvent	17I-28003	SM18_17I-28003_D01_UV-metric psKa_report.pdf
SM19	9.59			UV-metric pK <sub>a</sub> with cosolvent	17I-28004	SM19_17I-28004_D02_UV-metric psKa_report.pdf
SM19	9.58			UV-metric pK <sub>a</sub> with cosolvent	17I-28005	SM19_17I-28005_D02_UV-metric psKa_report.pdf
SM19	9.52			UV-metric pK <sub>a</sub> with cosolvent	17I-28006	SM19_17I-28006_D02_UV-metric psKa_report.pdf
SM20	5.72			UV-metric pK <sub>a</sub> with cosolvent	17J-12002	SM20_17J-12002_D05_UV-metric psKa_report.pdf
SM20	5.75			UV-metric pK <sub>a</sub> with cosolvent	17J-12003	SM20_17J-12003_D05_UV-metric psKa_report.pdf
SM20	5.64			UV-metric pK <sub>a</sub> with cosolvent	17K-16013	SM20_17K-16013_D05_UV-metric psKa_report.pdf
SM21	4.11			UV-metric pK <sub>a</sub> with cosolvent	17J-06002	SM21_17J-06002_D06_UV-metric psKa_report.pdf
SM21	4.09			UV-metric pK <sub>a</sub> with cosolvent	17J-06003	SM21_17J-06003_D06_UV-metric psKa_report.pdf
SM21	4.09			UV-metric pK <sub>a</sub> with cosolvent	17J-06004	SM21_17J-06004_D06_UV-metric psKa_report.pdf
SM22	2.45	7.42		UV-metric pK <sub>a</sub> with cosolvent	17J-07003	SM22_17J-07003_D07_UV-metric psKa_report.pdf
SM22	2.37	7.45		UV-metric pK <sub>a</sub> with cosolvent	17J-07004	SM22_17J-07004_D07_UV-metric psKa_report.pdf
SM22	2.37	7.41		UV-metric pK <sub>a</sub> with cosolvent	17J-07005	SM22_17J-07005_D07_UV-metric psKa_report.pdf
SM23	5.45			UV-metric pK <sub>a</sub> with cosolvent	17J-07006	SM23_17J-07006_D08_UV-metric psKa_report.pdf
SM23	5.44			UV-metric pK <sub>a</sub> with cosolvent	17J-07007	SM23_17J-07007_D08_UV-metric psKa_report.pdf
SM23	5.45			UV-metric pK <sub>a</sub> with cosolvent	17J-07008	SM23_17J-07008_D08_UV-metric psKa_report.pdf
SM24	2.61			UV-metric pK <sub>a</sub> with cosolvent	17J-06007	SM24_17J-06007_D09_UV-metric psKa_report.pdf
SM24	2.58			UV-metric pK <sub>a</sub> with cosolvent	17J-06008	SM24_17J-06008_D09_UV-metric psKa_report.pdf
SM24	2.6			UV-metric pK <sub>a</sub> with cosolvent	17J-06009	SM24_17J-06009_D09_UV-metric psKa_report.pdf

**Table SI 4.** UV-metric pK<sub>a</sub> measurements with and without cosolvent for 12 pK<sub>a</sub> challenge compounds with higher aqueous solubility and pyridoxine HCl. pK<sub>a</sub> values were measured with both methods to test if cosolvent method introduces bias.

Molecule ID	pK <sub>a1</sub>	pK <sub>a2</sub>	pK <sub>a3</sub>	Assay Type	Experiment ID	Experiment Report
SM01	9.54			UV-metric pK <sub>a</sub>	17I-15024	SM01_17I-15024_M01_UV-metric pKa_report.pdf
SM01	9.53			UV-metric pK <sub>a</sub>	17I-15025	SM01_17I-15025_M01_UV-metric pKa_report.pdf
SM01	9.53			UV-metric pK <sub>a</sub>	17I-16001	SM01_17I-16001_M01_UV-metric pKa_report.pdf
SM01*	9.71			UV-metric pK <sub>a</sub> with cosolvent	17J-06011	SM01_17J-06011_M01_UV-metric psKa_report.pdf
SM04	6.03			UV-metric pK <sub>a</sub>	17I-18018	SM04_17I-18018_M04_UV-metric pKa_report.pdf
SM04	6.02			UV-metric pK <sub>a</sub>	17I-18019	SM04_17I-18019_M04_UV-metric pKa_report.pdf
SM04	6.02			UV-metric pK <sub>a</sub>	17I-18020	SM04_17I-18020_M04_UV-metric pKa_report.pdf
SM04*	5.97			UV-metric pK <sub>a</sub> with cosolvent	17K-09020	SM04_17K-09020_M04_UV-metric psKa_report.pdf
SM06	2.96	11.76		UV-metric pK <sub>a</sub>	17I-18021	SM06_17I-18021_M06_UV-metric pKa_report.pdf
SM06	3.11	11.74		UV-metric pK <sub>a</sub>	17I-18022	SM06_17I-18022_M06_UV-metric pKa_report.pdf
SM06	3.02	11.71		UV-metric pK <sub>a</sub>	17I-18023	SM06_17I-18023_M06_UV-metric pKa_report.pdf
SM06*	3.46	>12		UV-metric pK <sub>a</sub> with cosolvent	17K-09021	SM06_17K-09021_M06_UV-metric psKa_report.pdf
SM06*	3.4	>12		UV-metric pK <sub>a</sub> with cosolvent	17K-10008	SM06_17K-10008_M06_UV-metric psKa_report.pdf
SM06*	3.45	>12		UV-metric pK <sub>a</sub> with cosolvent	17K-16011	SM06_17K-16011_M06_UV-metric psKa_report.pdf
SM07	6.07			UV-metric pK <sub>a</sub>	17I-16002	SM07_17I-16002_M07_UV-metric pKa_report.pdf
SM07	6.07			UV-metric pK <sub>a</sub>	17I-16004	SM07_17I-16004_M07_UV-metric pKa_report.pdf
SM07	6.09			UV-metric pK <sub>a</sub>	17I-20001	SM07_17I-20001_M07_UV-metric pKa_report.pdf
SM07*	5.96			UV-metric pK <sub>a</sub> with cosolvent	17J-12006	SM07_17J-12006_M07_UV-metric psKa_report.pdf
SM08	4.23			UV-metric pK <sub>a</sub>	17I-19001	SM08_17I-19001_M08_UV-metric pKa_report.pdf
SM08	4.2			UV-metric pK <sub>a</sub>	17I-19002	SM08_17I-19002_M08_UV-metric pKa_report.pdf
SM08	4.22			UV-metric pK <sub>a</sub>	17I-19003	SM08_17I-19003_M08_UV-metric pKa_report.pdf
SM08*	4.47			UV-metric pK <sub>a</sub> with cosolvent	17J-12007	SM08_17J-12007_M08_UV-metric psKa_report.pdf
SM11	3.89			UV-metric pK <sub>a</sub>	17I-16005	SM11_17I-16005_M11_UV-metric pKa_report.pdf
SM11	3.89			UV-metric pK <sub>a</sub>	17I-16006	SM11_17I-16006_M11_UV-metric pKa_report.pdf
SM11	3.89			UV-metric pK <sub>a</sub>	17I-16007	SM11_17I-16007_M11_UV-metric pKa_report.pdf
SM11*	3.74			UV-metric pK <sub>a</sub> with cosolvent	17J-04003	SM11_17J-04003_M11_UV-metric psKa_report.pdf
SM12	5.28			UV-metric pK <sub>a</sub>	17I-21002	SM12_17I-21002_M12_UV-metric pKa_report.pdf
SM12	5.28			UV-metric pK <sub>a</sub>	17I-21003	SM12_17I-21003_M12_UV-metric pKa_report.pdf
SM12	5.27			UV-metric pK <sub>a</sub>	17I-21004	SM12_17I-21004_M12_UV-metric pKa_report.pdf
SM12*	5.16			UV-metric pK <sub>a</sub> with cosolvent	17J-11014	SM12_17J-11014_M12_UV-metric psKa_report.pdf
SM13	5.79			UV-metric pK <sub>a</sub>	17I-16011	SM13_17I-16011_M13_UV-metric pKa_report.pdf
SM13	5.76			UV-metric pK <sub>a</sub>	17I-16012	SM13_17I-16012_M13_UV-metric pKa_report.pdf
SM13	5.76			UV-metric pK <sub>a</sub>	17I-16013	SM13_17I-16013_M13_UV-metric pKa_report.pdf
SM13*	5.68			UV-metric pK <sub>a</sub> with cosolvent	17J-04005	SM13_17J-04005_M13_UV-metric psKa_report.pdf
SM14	2.59	5.31		UV-metric pK <sub>a</sub>	17I-29002	SM14_17I-29002_M15_UV-metric pKa_report.pdf
SM14	2.57	5.29		UV-metric pK <sub>a</sub>	17I-29003	SM14_17I-29003_M15_UV-metric pKa_report.pdf
SM14	2.57	5.29		UV-metric pK <sub>a</sub>	17I-29004	SM14_17I-29004_M15_UV-metric pKa_report.pdf
SM14*	2.56	5.29		UV-metric pK <sub>a</sub> with cosolvent	17J-12008	SM14_17J-12008_M15_UV-metric psKa_report.pdf

\* These UV-metric pK<sub>a</sub> measurement with cosolvent were not used in the calculation of pK<sub>a</sub> mean and SEM in the experimental data reported for SAMPL6 pK<sub>a</sub> challenge because replicate water based experiments also existed for these molecules.

**Table SI 4.** Continued.

Molecule ID	pK <sub>a1</sub>	pK <sub>a2</sub>	pK <sub>a3</sub>	Assay Type	Experiment ID	Experiment Report
SM15	4.71	8.96		UV-metric pK <sub>a</sub>	17K-10009	SM15_17K-10009_M16_UV-metric pKa_report.pdf
SM15	4.7	8.94		UV-metric pK <sub>a</sub>	17K-10010	SM15_17K-10010_M16_UV-metric pKa_report.pdf
SM15	4.69	8.92		UV-metric pK <sub>a</sub>	17K-10011	SM15_17K-10011_M16_UV-metric pKa_report.pdf
SM15*	4.67	9.01		UV-metric pK <sub>a</sub> with cosolvent	17J-12009	SM15_17J-12009_M16_UV-metric psKa_report.pdf
SM16	5.37	10.64		UV-metric pK <sub>a</sub>	17J-03025	SM16_17J-03025_M18_UV-metric pKa_report.pdf
SM16	5.37	10.65		UV-metric pK <sub>a</sub>	17J-03026	SM16_17J-03026_M18_UV-metric pKa_report.pdf
SM16	5.38	10.65		UV-metric pK <sub>a</sub>	17J-03027	SM16_17J-03027_M18_UV-metric pKa_report.pdf
SM16*	5.35	11.01		UV-metric pK <sub>a</sub> with cosolvent	17J-12010	SM16_17J-12010_M18_UV-metric psKa_report.pdf
SM17	3.15			UV-metric pK <sub>a</sub>	17J-02024	SM17_17J-02024_M19_UV-metric pKa_report.pdf
SM17	3.16			UV-metric pK <sub>a</sub>	17J-02025	SM17_17J-02025_M19_UV-metric pKa_report.pdf
SM17	3.17			UV-metric pK <sub>a</sub>	17J-02026	SM17_17J-02026_M19_UV-metric pKa_report.pdf
SM17*	3.2			UV-metric pK <sub>a</sub> with cosolvent	17J-12011	SM17_17J-12011_M19_UV-metric psKa_report.pdf
Pyridoxine HCl	4.81	8.85		UV-metric pK <sub>a</sub>	18E-22003	18E-22003_Pyridoxine HCl_UV-metric pKa_0417936-0002.pdf
Pyridoxine HCl	4.83	8.87		UV-metric pK <sub>a</sub>	18E-22004	18E-22004_Pyridoxine HCl_UV-metric pKa_0417936-0002.pdf
Pyridoxine HCl	4.84	8.86		UV-metric pK <sub>a</sub>	18E-22010	18E-22010_Pyridoxine HCl_UV-metric pKa_0417936-0002.pdf
Pyridoxine HCl	4.85	8.79		UV-metric pK <sub>a</sub> with cosolvent	18E-22011	18E-22011_Pyridoxine HCl_UV-metric psKa_0417936-0002.pdf
Pyridoxine HCl	4.85	8.79		UV-metric pK <sub>a</sub> with cosolvent	18E-22012	18E-22012_Pyridoxine HCl_UV-metric psKa_0417936-0002.pdf
Pyridoxine HCl	4.86	8.77		UV-metric pK <sub>a</sub> with cosolvent	18E-22013	18E-22013_Pyridoxine HCl_UV-metric psKa_0417936-0002.pdf

\* These UV-metric pK<sub>a</sub> measurement with cosolvent were not used in the calculation of pK<sub>a</sub> mean and SEM in the experimental data reported for SAMPL6 pK<sub>a</sub> challenge because replicate water based experiments also existed for these molecules.

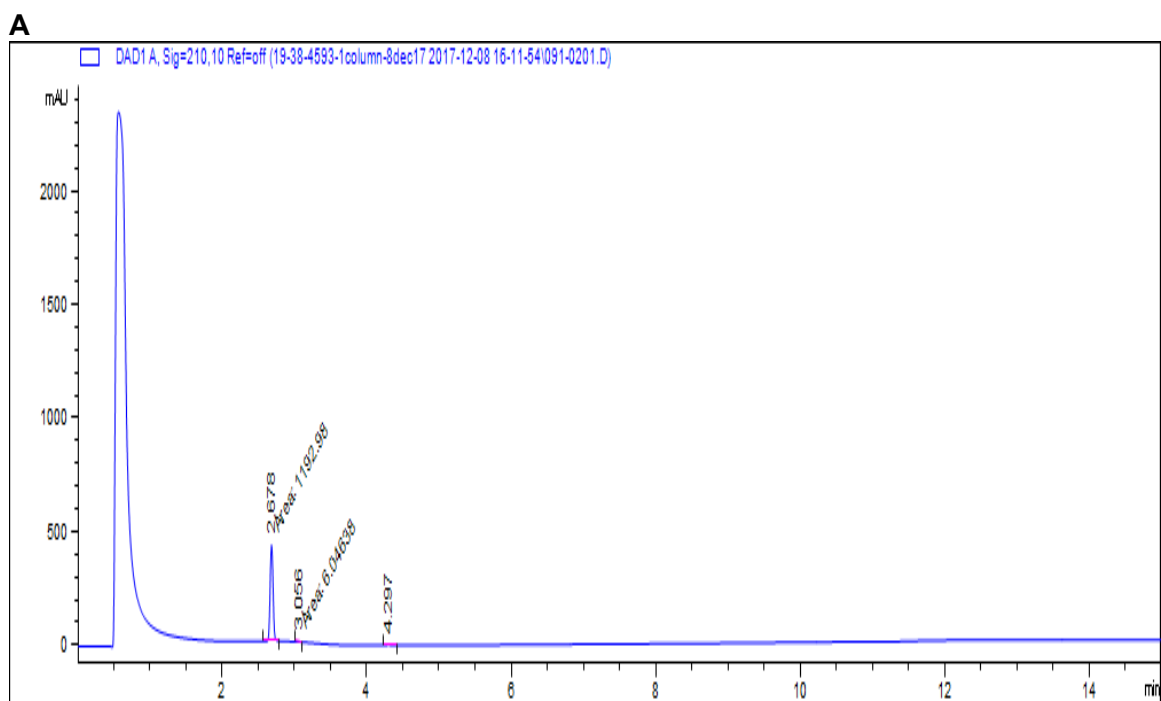
**Table SI 5.** pK<sub>a</sub> mean and SEM results of with (cosolvent) and without cosolvent (water) replicate experiment. pK<sub>a</sub> values without SEM reported were measured with 1 replicate. pK<sub>a</sub> values reported with SEM were measured in triplicates.

Molecule ID	pK <sub>a</sub> ID	pK <sub>a</sub> mean water	pK <sub>a</sub> SEM water	pK <sub>a</sub> mean cosolvent	pK <sub>a</sub> SEM cosolvent
SM01	SM01_pKa1	9.53	0.01	9.71	
SM04	SM04_pKa1	6.02	0.01	5.97	
SM06	SM06_pKa1	3.03	0.04	3.44	0.02
SM07	SM07_pKa1	6.08	0.01	5.96	
SM08	SM08_pKa1	4.22	0.01	4.47	
SM11	SM11_pKa1	3.89	0.01	3.74	
SM12	SM12_pKa1	5.28	0.01	5.16	
SM13	SM13_pKa1	5.77	0.01	5.68	
SM14	SM14_pKa1	2.58	0.01	2.56	
SM14	SM14_pKa2	5.3	0.01	5.29	
SM15	SM15_pKa1	4.7	0.01	4.67	
SM15	SM15_pKa2	8.94	0.01	9.01	
SM16	SM16_pKa1	5.37	0.01	5.35	
SM16	SM16_pKa2	10.65	0.01	11.01	
SM17	SM17_pKa1	3.16	0.01	3.2	
Pyridoxine HCl	Pyridoxine HCl_pKa1	4.83	0.01	4.85	0.01
Pyridoxine HCl	Pyridoxine HCl_pKa2	8.86	0.01	8.78	0.01

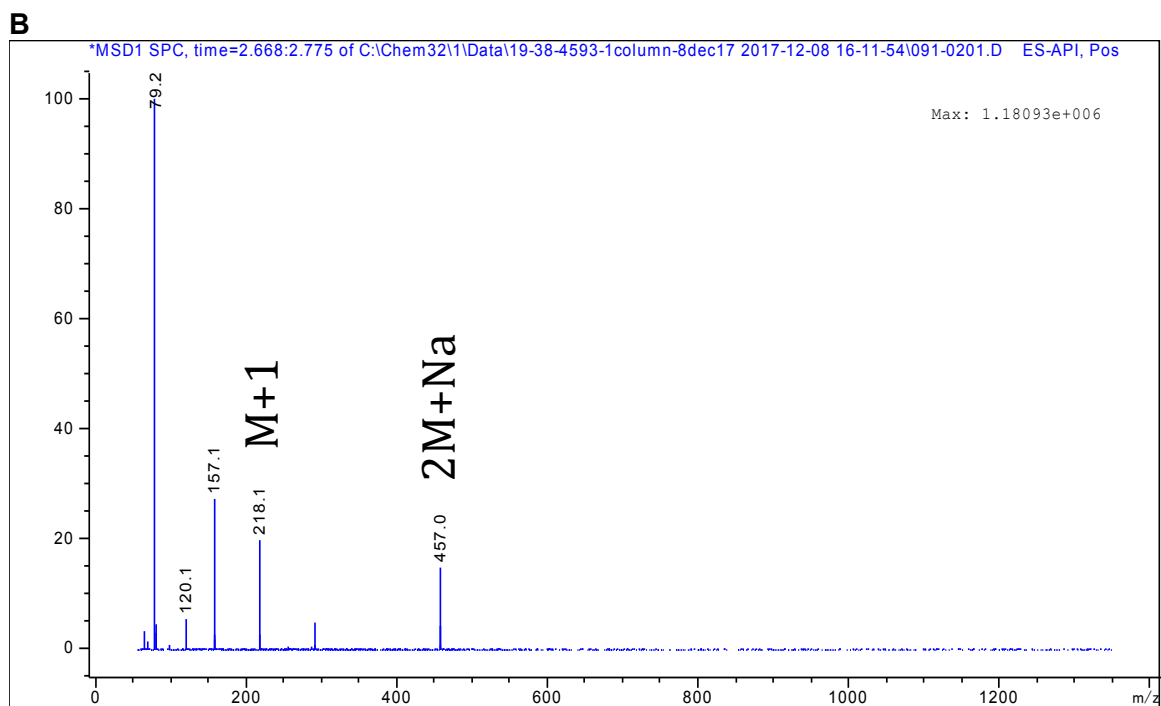


**Table SI 6.** Summary of LC-MS purity results of SAMPL6 pK<sub>a</sub> challenge compounds. LC-MS purity was calculated as percentage of area under the curve relative to all the peaks observed.

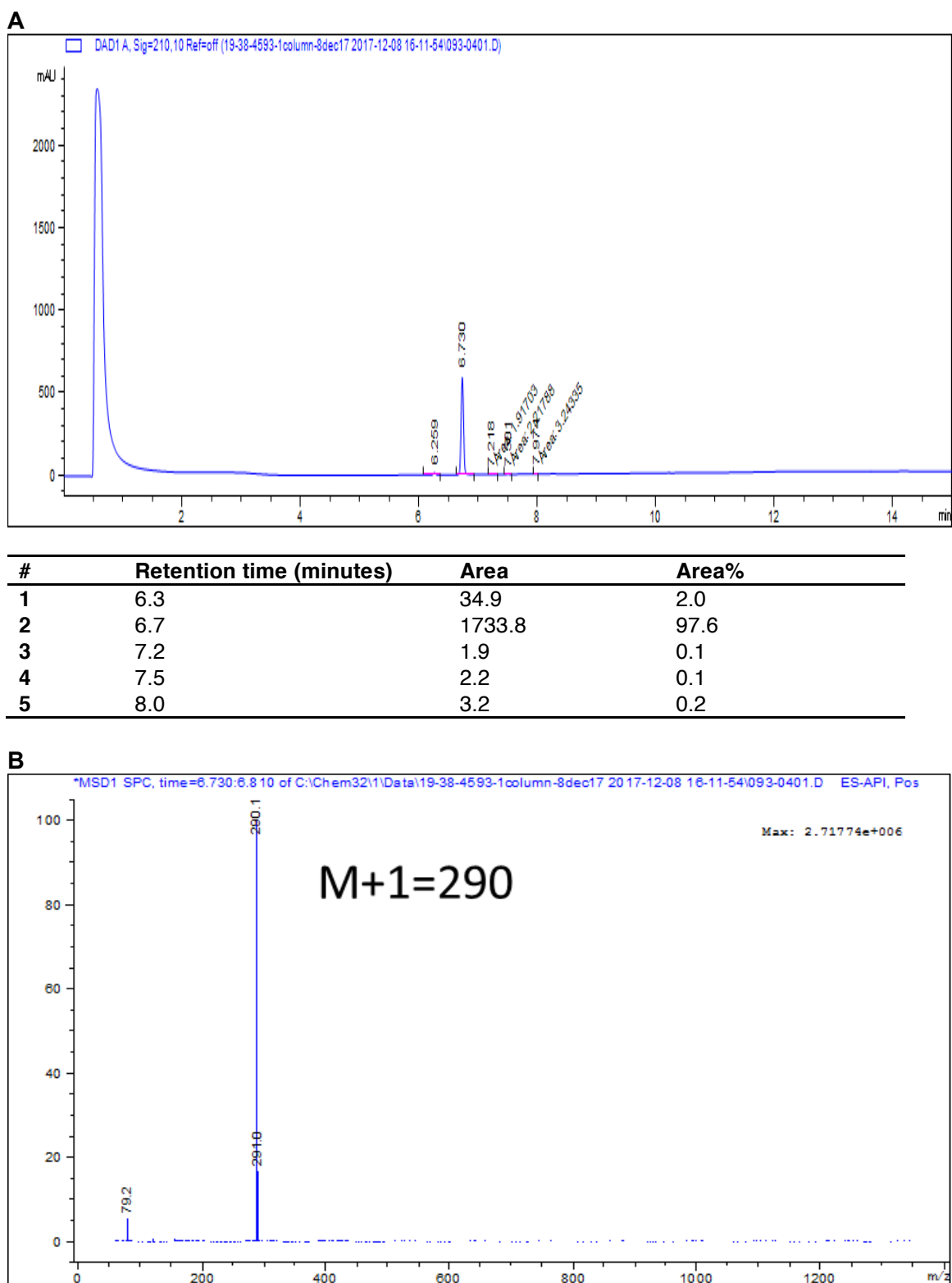
SAMPL6 molecule ID	Supplier reported purity (%)	LC-MS purity (Area%)	Supplier reported molecular weight (g/mol)	eMolecules reported molecular weight (g/mol)	LC/MS molecular weight (g/mol)	eMolecules ID
SM01	95	98.516	217.23	217.221	217.221	6679830
SM02		97.62		289.255	289.255	1327907
SM03		90.798		301.387	301.387	1228629
SM04		99.884		269.729	269.729	30719859
SM05		98.749		304.771	304.771	18908671
SM06		98.913		328.163	328.163	18893169
SM07	95	99.384	235.284	235.284	235.284	1327878
SM08	95	99.242	293.317	293.317	293.317	1367649
SM09	95	98.969	287.744	287.744	287.744	1865544
SM10	95	96.98	311.357	311.358	311.358	23354217
SM11	90	98.022	267.114	211.223	211.233	719540
SM12		98.134		292.163	292.163	1859493
SM13		97.512		295.336	295.336	5828805
SM14		96.484		209.247	209.247	31653344
SM15		98.698		210.231	210.231	37095168
SM16		97.733		267.111	267.111	1284691
SM17		96.994	380.25	269.322	269.322	1444229
SM18		92.601		426.439	426.439	18897105
SM19		94.464		381.276	381.276	3365457
SM20		97.176		380.245	380.245	46568819
SM21		96.791		438.092	438.092	1574612
SM22		86.975		396.951	396.951	536848
SM23		96.011	420.47	420.461	420.461	4375254
SM24		99.331	391.43	391.42	391.42	1415746



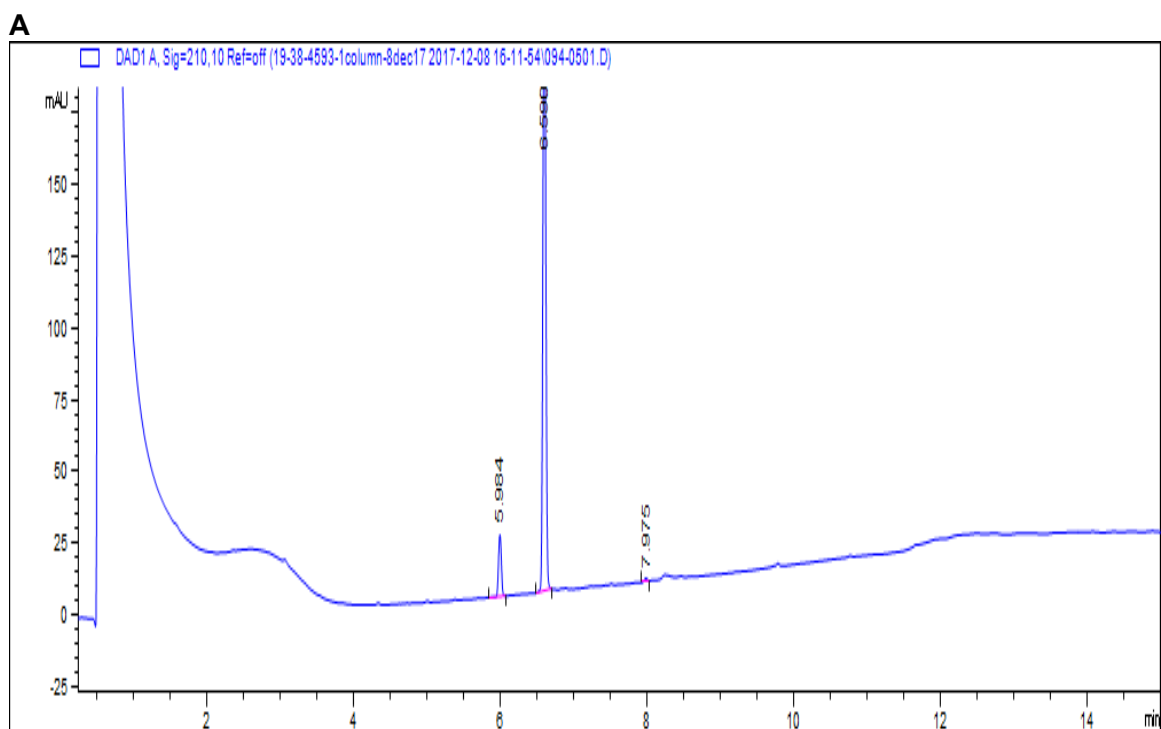
#	Retention time (minutes)	Area	Area%
1	2.7	1193	98.5
2	3.1	6	0.5
3	4.3	11.9	1.0



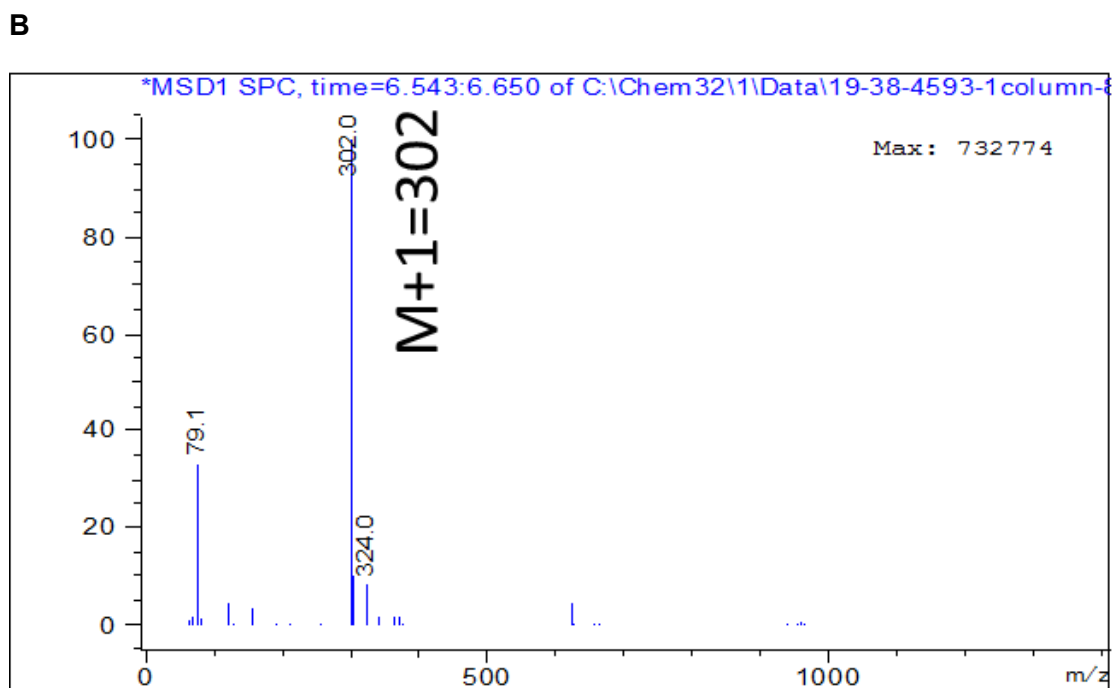
**Figure SI 1.** LC-MS chromatogram and mass spectrum for SM01. Molecular weight is 217.221 g/mol. (A) LC chromatogram and table of peak properties. %Area describes relative area under the curve of selected peaks and interpreted as an estimate of purity. (B) Mass spectrum



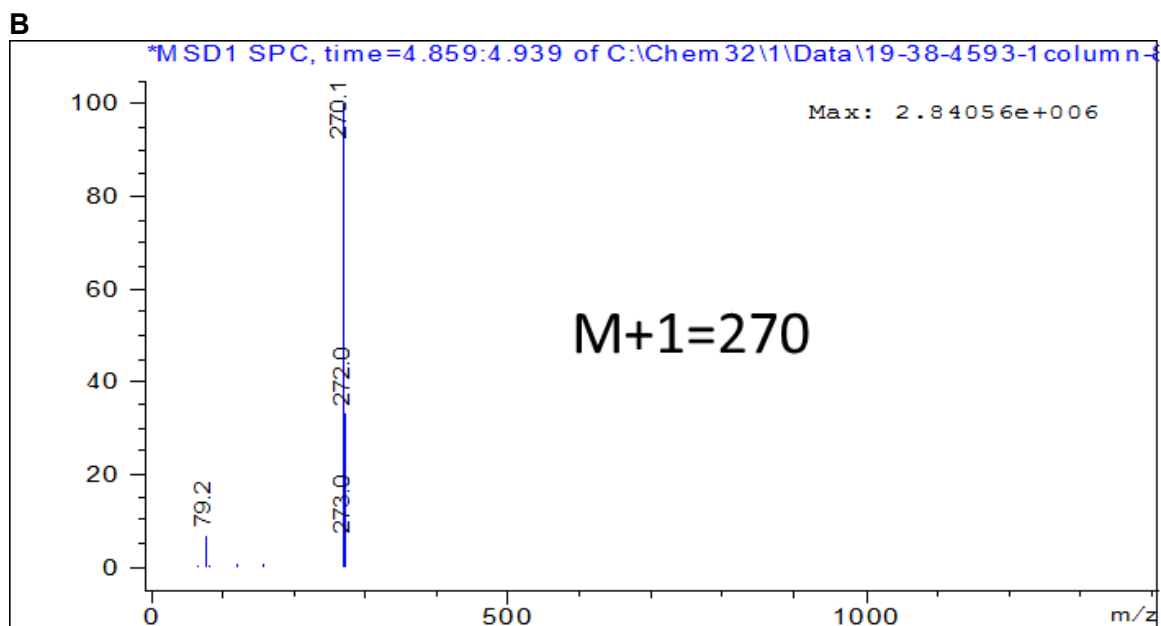
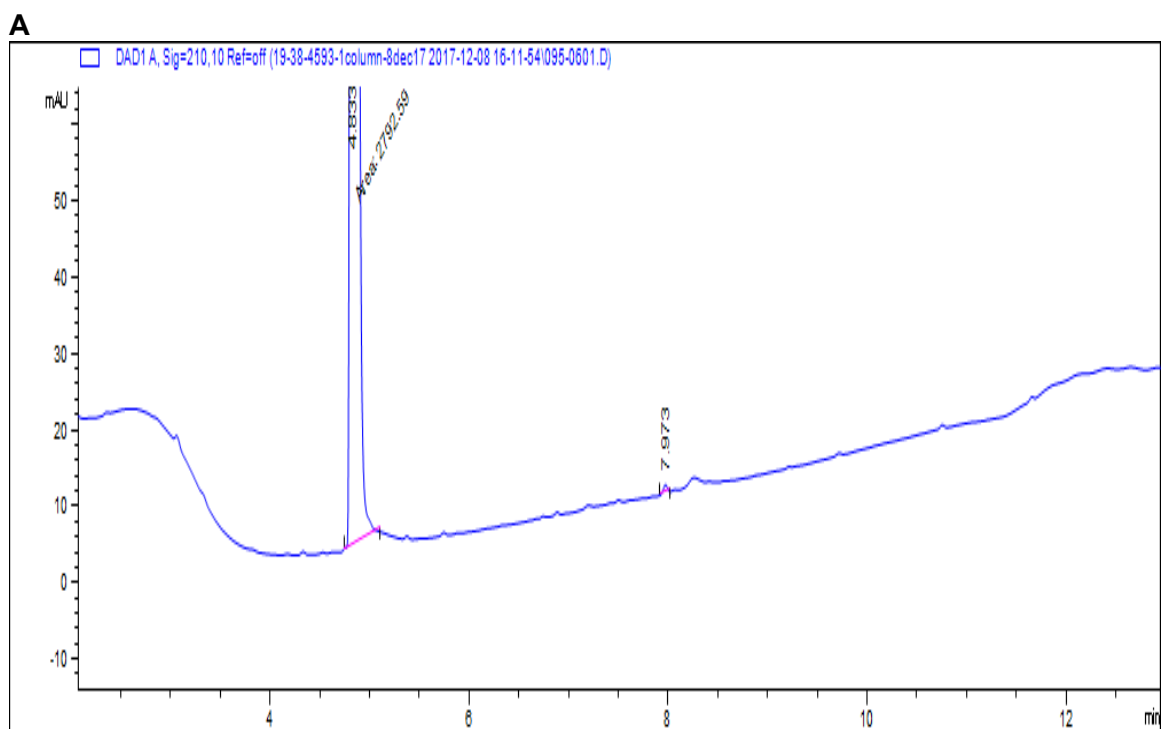
**Figure SI 2.** LC-MS chromatogram and mass spectrum for SM02. Molecular weight is 289.255 g/mol. (A) LC chromatogram and table of peak properties. %Area describes relative area under the curve of selected peaks and interpreted as an estimate of purity. (B) Mass spectrum



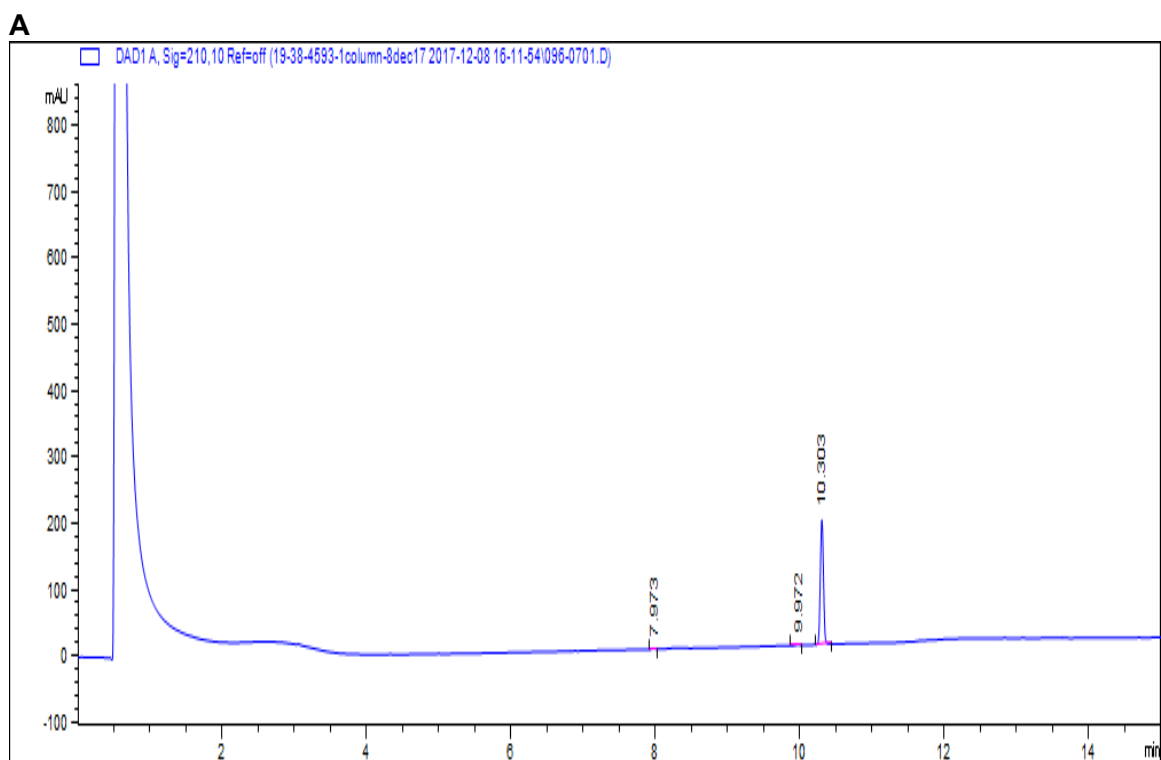
#	Retention time (minutes)	Area	Area%
1	6.0	58.2	8.7
2	6.6	606.7	90.8
3	8.0	3.3	0.5



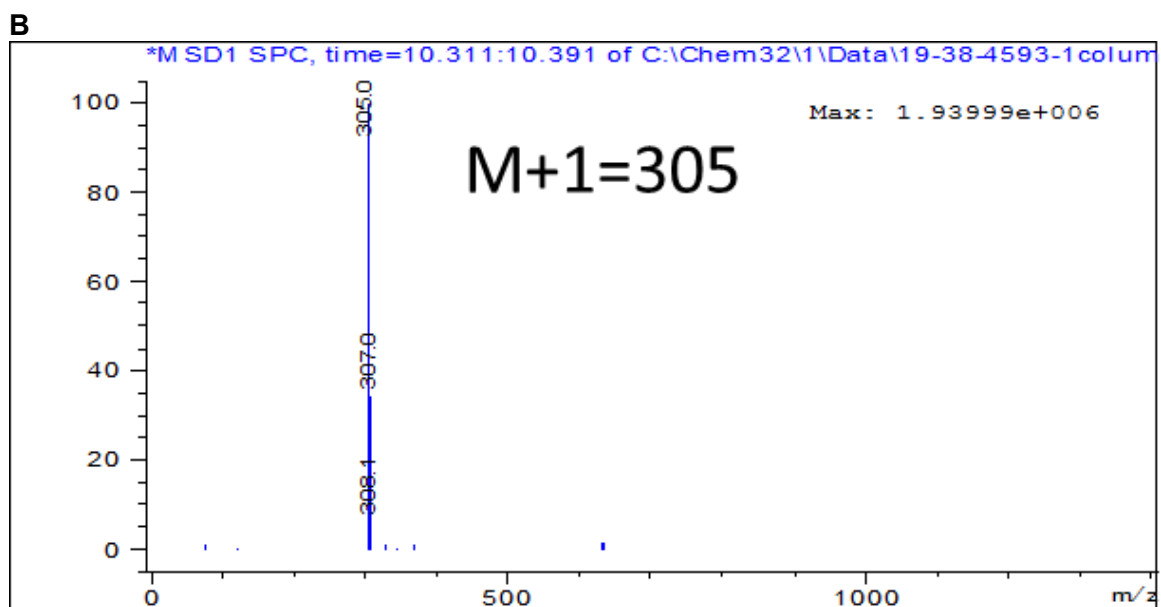
**Figure SI 3.** LC-MS chromatogram and mass spectrum for SM03. Molecular weight is 301.387 g/mol. (A) LC chromatogram and table of peak properties. %Area describes relative area under the curve of selected peaks and interpreted as an estimate of purity. (B) Mass spectrum



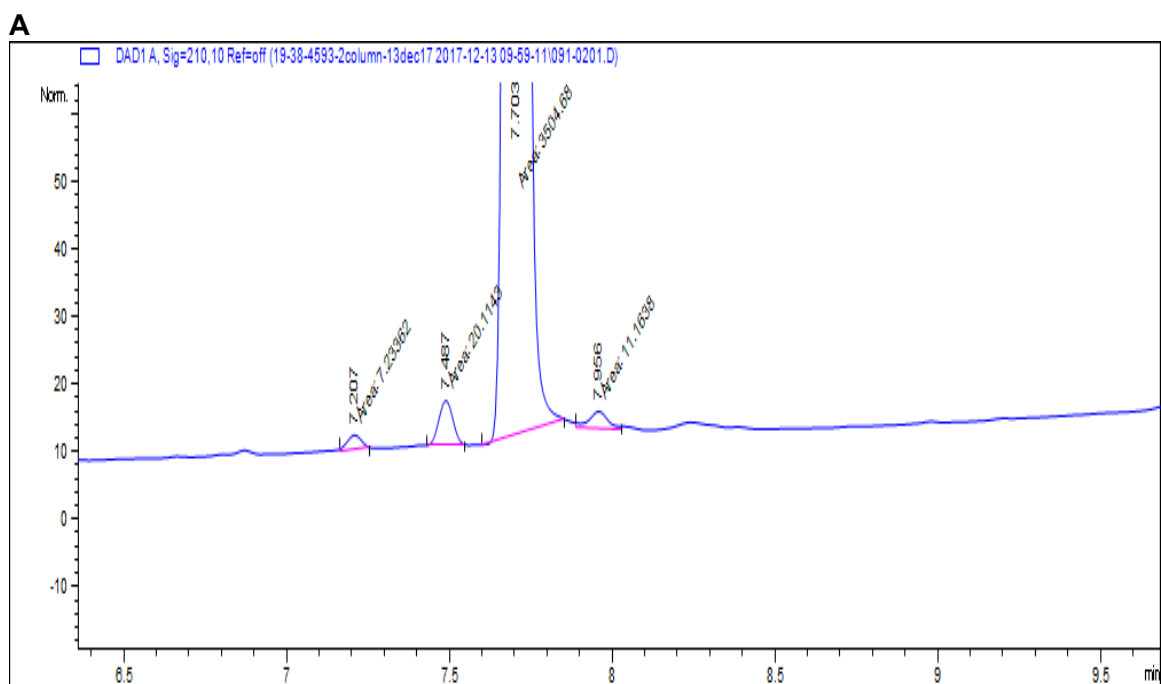
**Figure SI 4.** LC-MS chromatogram and mass spectrum for SM04. Molecular weight is 269.729 g/mol. (A) LC chromatogram and table of peak properties. %Area describes relative area under the curve of selected peaks and interpreted as an estimate of purity. (B) Mass spectrum



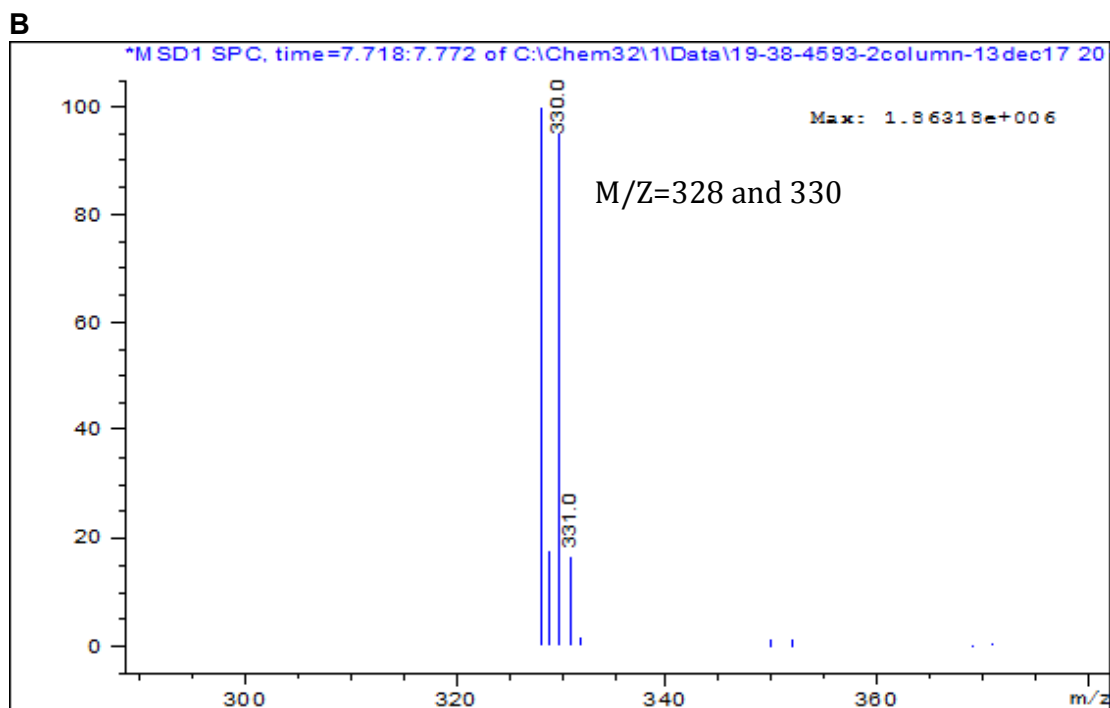
#	Retention time (minutes)	Area	Area%
1	8.0	3.3	0.6
2	10.0	4	0.7
3	10.3	569.7	98.7



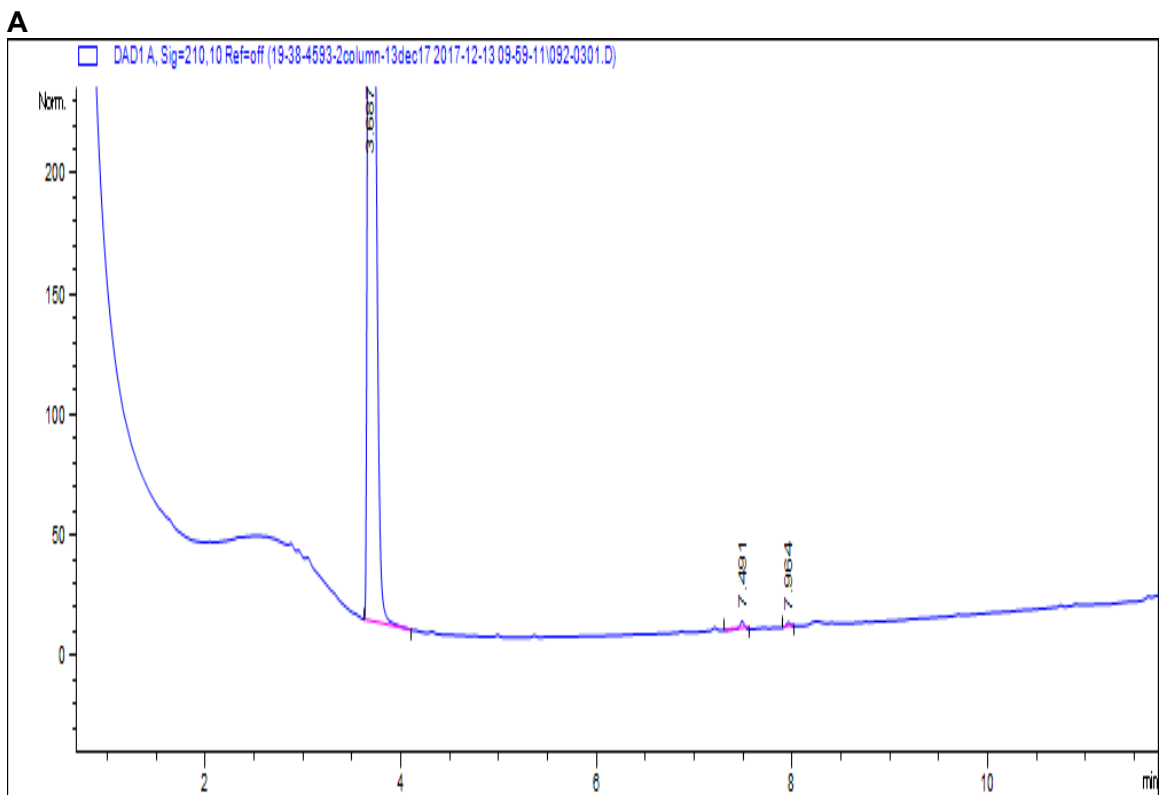
**Figure SI 5.** LC-MS chromatogram and mass spectrum for SM05. Molecular weight is 304.771 g/mol. (A) LC chromatogram and table of peak properties. %Area describes relative area under the curve of selected peaks and interpreted as an estimate of purity. (B) Mass spectrum



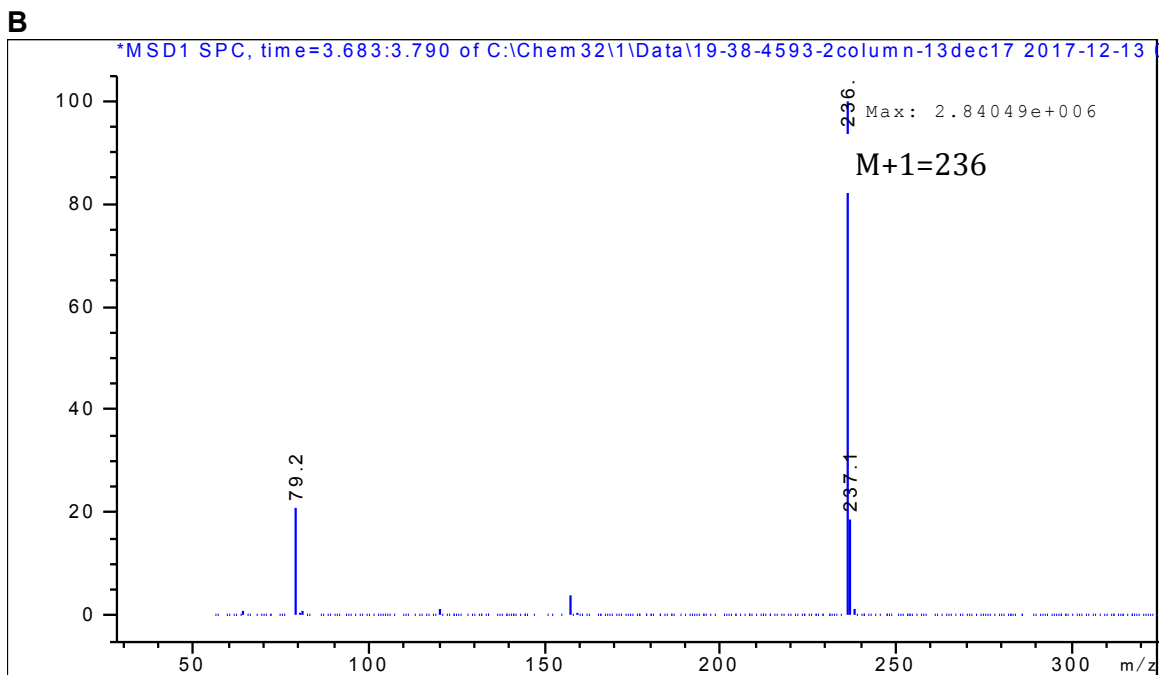
#	Retention time (minutes)	Area	Area%
1	7.2	7.2	0.2
2	7.5	20.1	0.6
3	7.7	3504.7	98.9
4	8.0	11.2	0.3



**Figure SI 6.** LC-MS chromatogram and mass spectrum for SM06. Molecular weight is 328.163 g/mol. (A) LC chromatogram and table of peak properties. %Area describes relative area under the curve of selected peaks and interpreted as an estimate of purity. (B) Mass spectrum

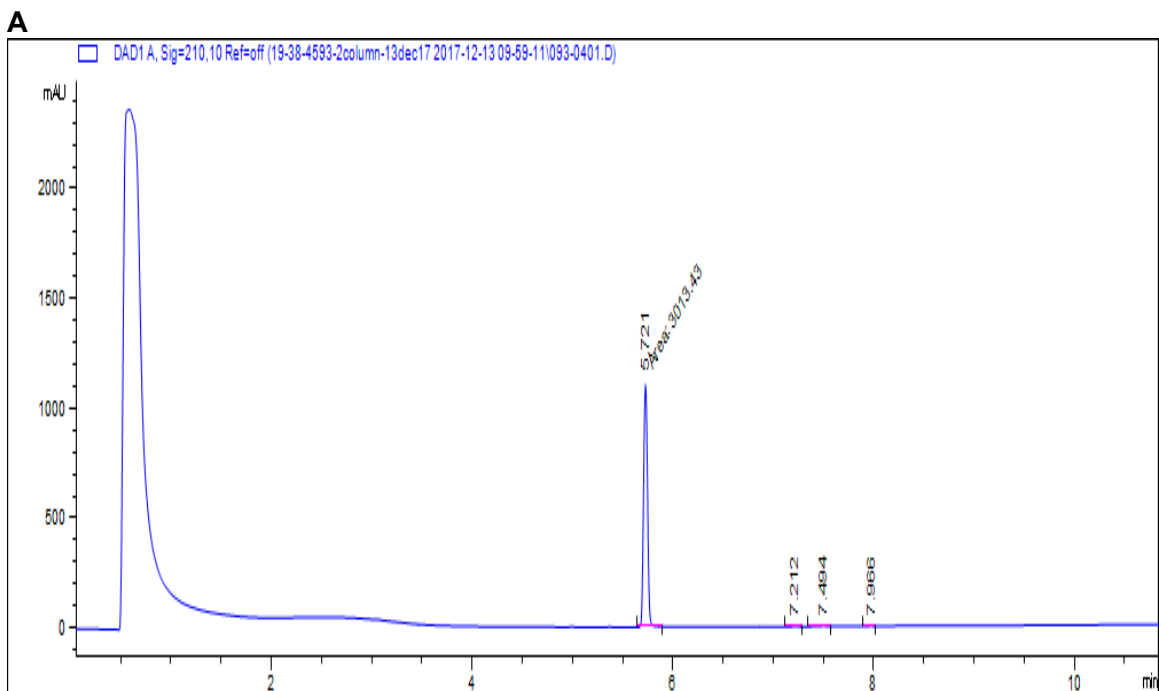


#	Retention time (minutes)	Area	Area%
1	3.7	2822.4	99.4
2	7.5	11.3	0.4
3	8.0	6.2	0.2

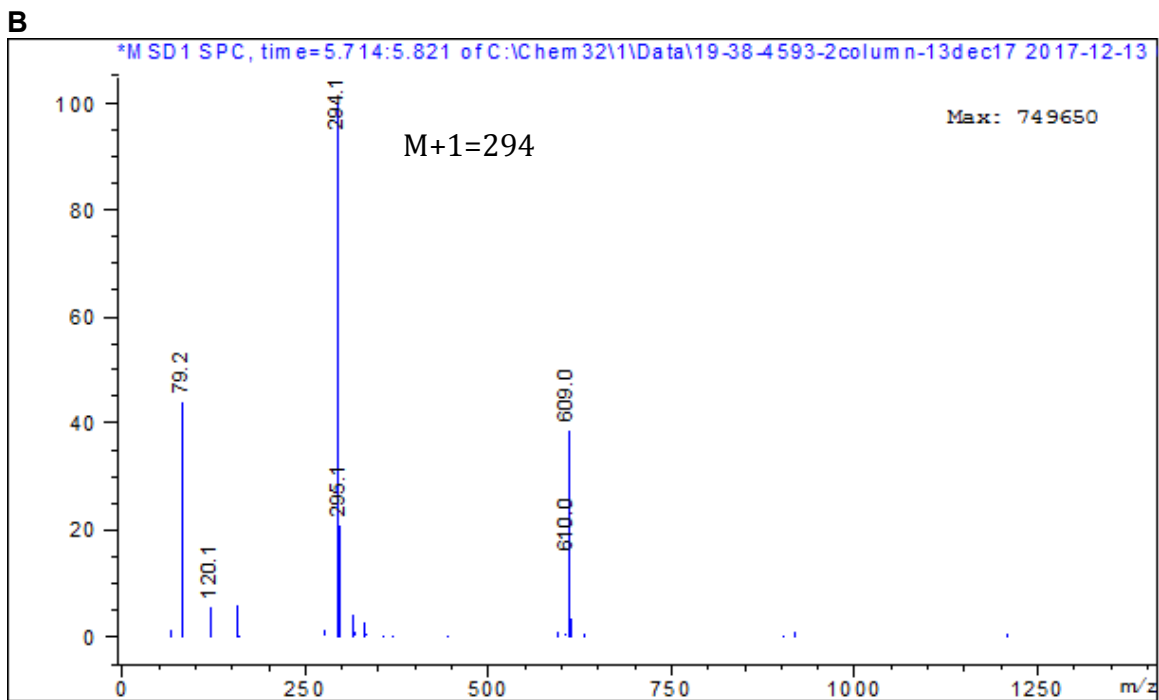


**Figure SI 7.** LC-MS chromatogram and mass spectrum for SM07. Molecular weight is 235.284 g/mol. (A) LC chromatogram and table of peak properties. %Area describes relative area under the curve of selected peaks and interpreted as an estimate of purity. (B) Mass spectrum

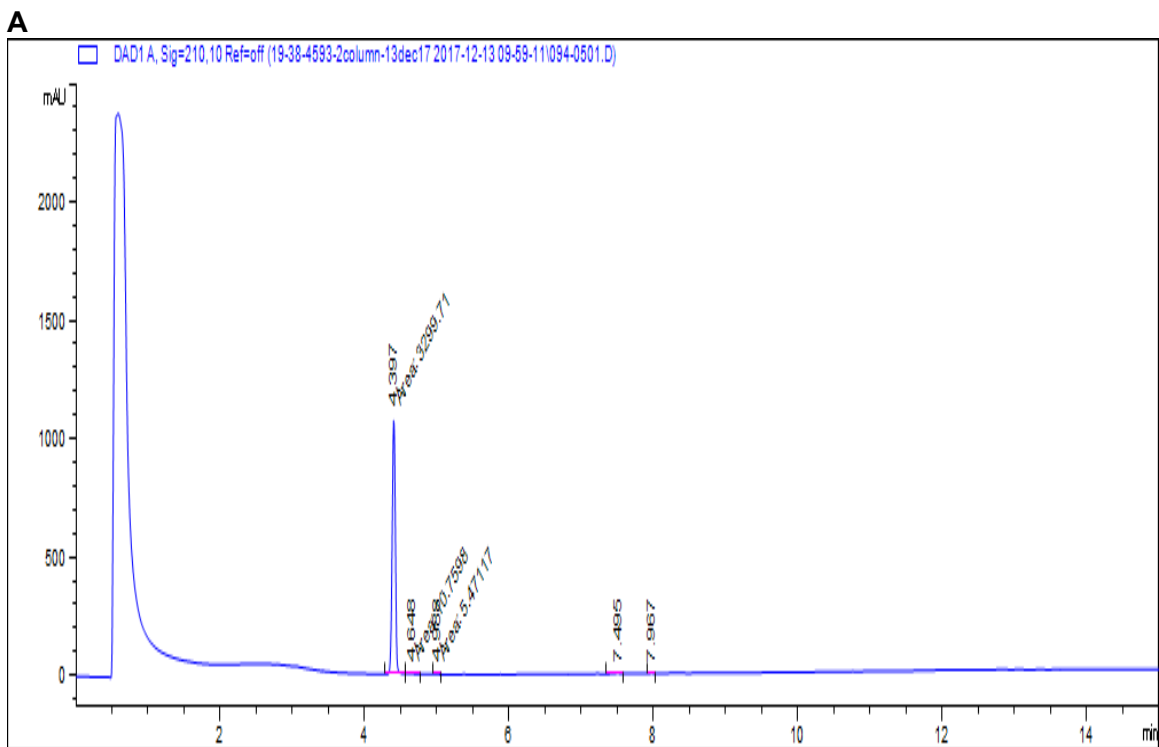




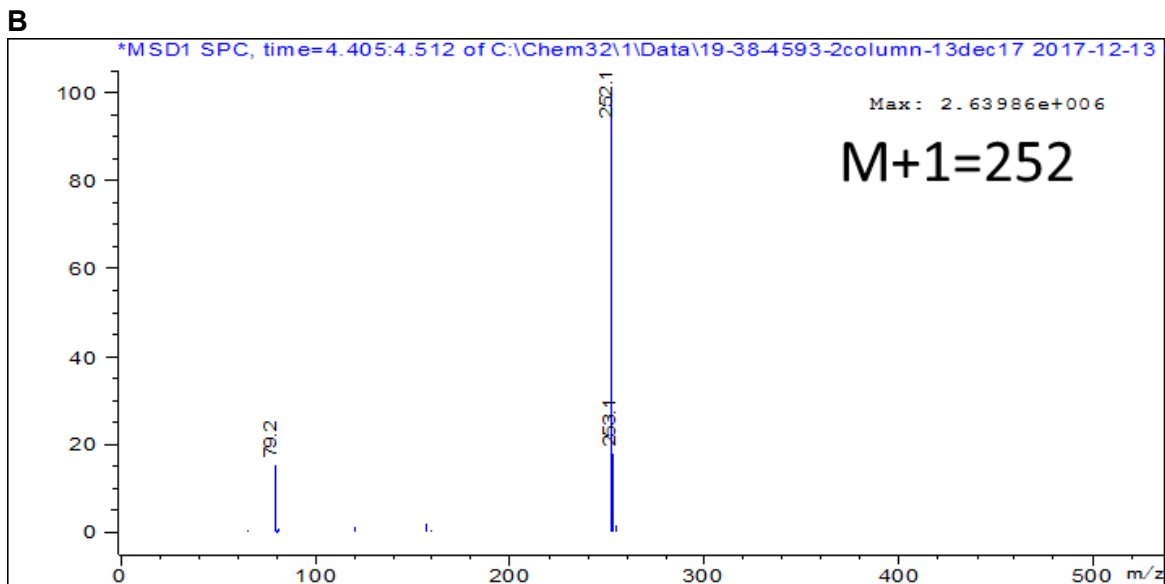
#	Retention time (minutes)	Area	Area%
1	5.7	3013.4	99.2
2	7.2	5	0.2
3	7.5	11.1	0.4
4	8.0	6.9	0.2



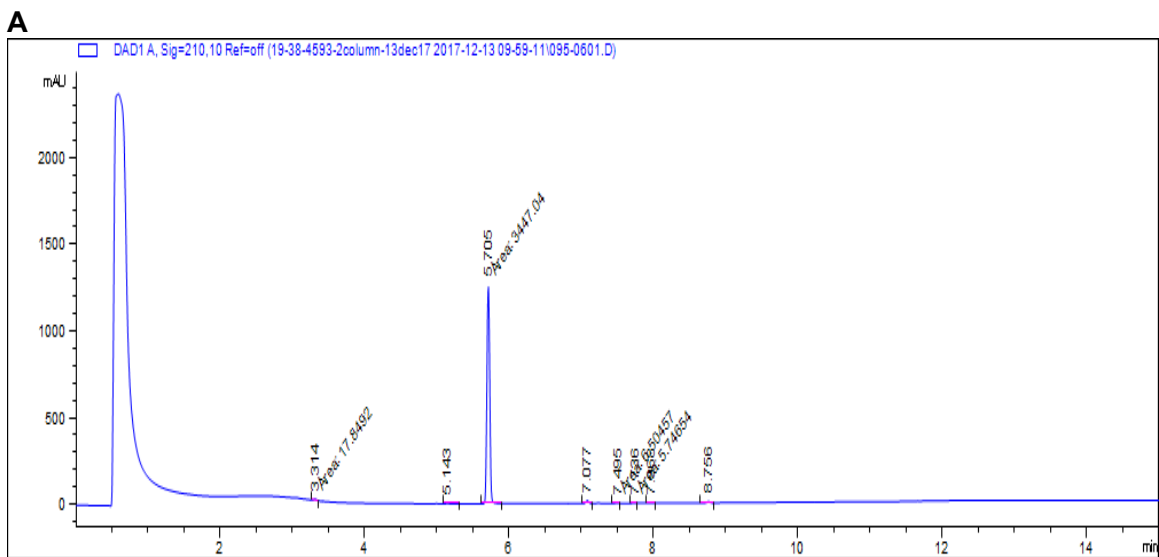
**Figure SI 8.** LC-MS chromatogram and mass spectrum for SM08. Molecular weight estimated from mass spectrometry is 293.317 g/mol. (A) LC chromatogram and table of peak properties. %Area describes relative area under the curve of selected peaks and interpreted as an estimate of purity. (B) Mass spectrum



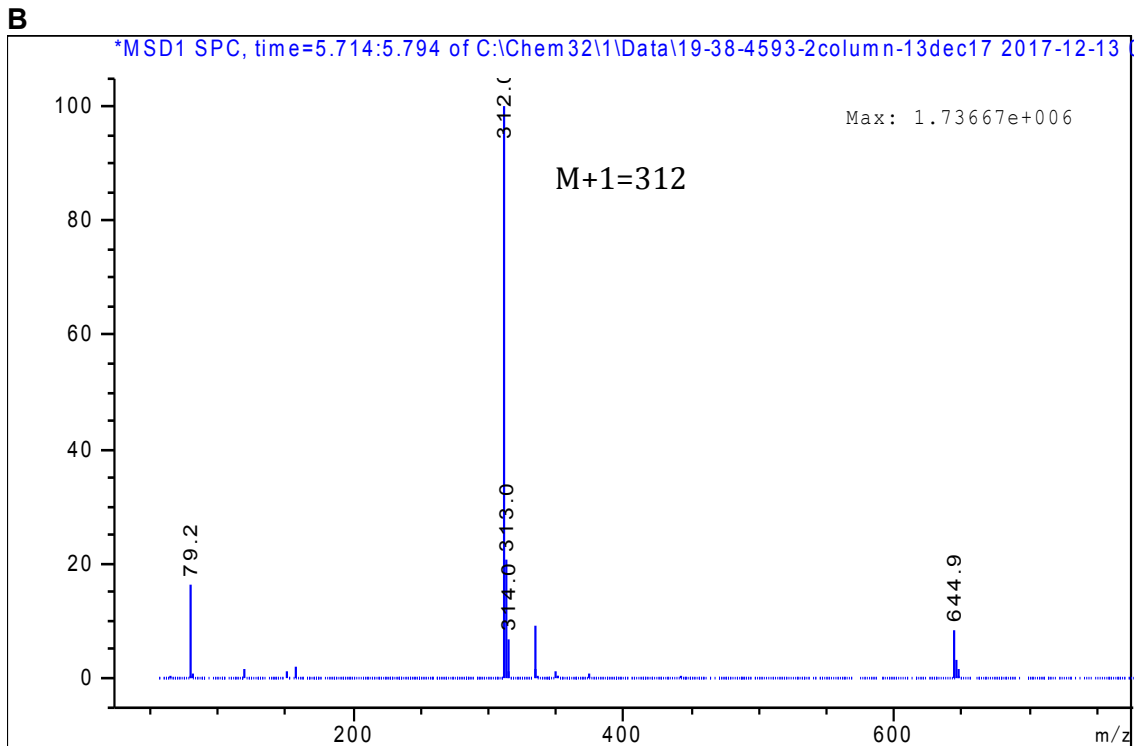
#	Retention time (minutes)	Area	Area%
1	4.4	3299.7	99.0
2	4.6	10.8	0.3
3	5.0	5.5	0.2
4	7.5	12.6	0.4
5	8.0	5.7	0.2



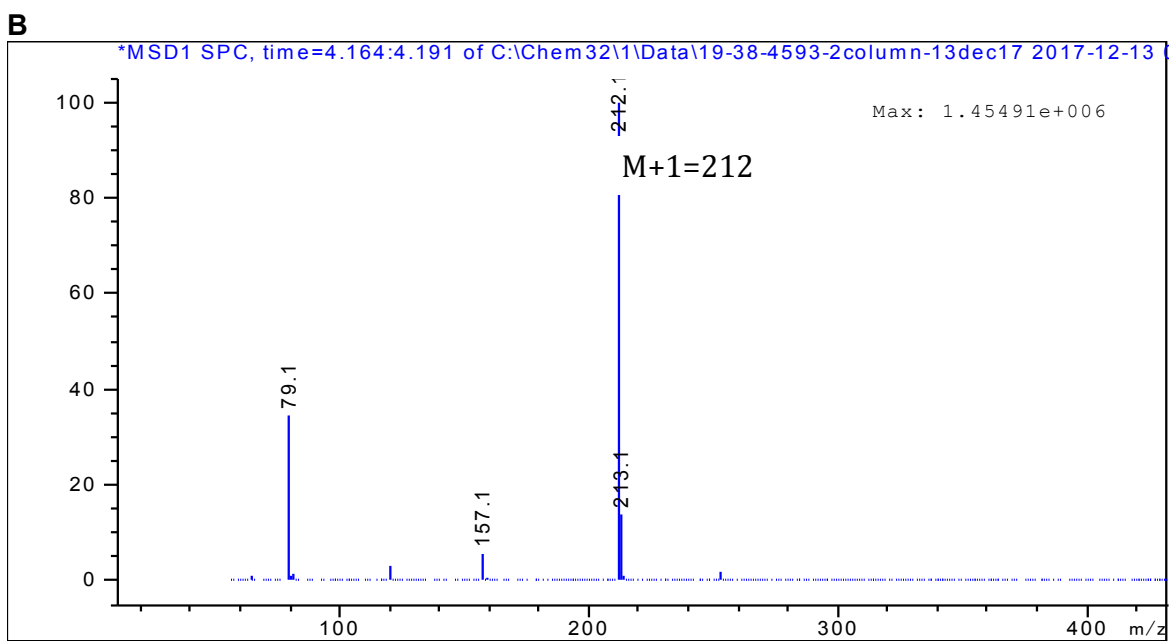
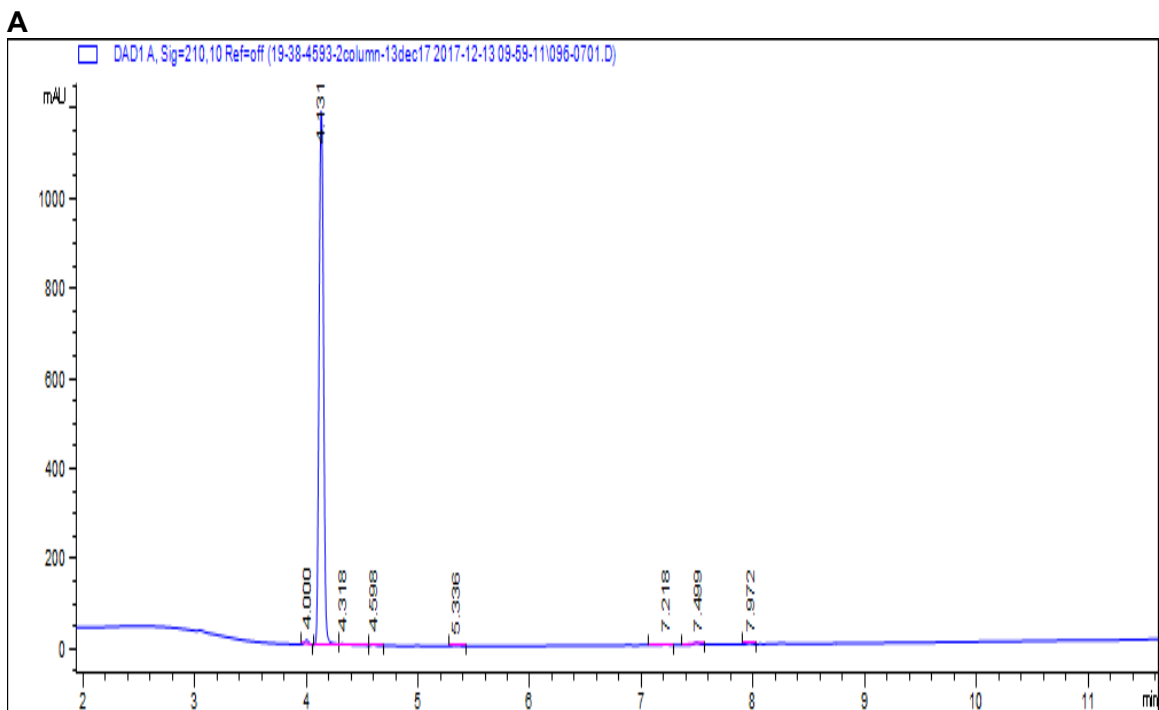
**Figure SI 9.** LC-MS chromatogram and mass spectrum for SM09. Molecular weight estimated from mass spectrometry is 287.744 g/mol ( $m/z = 251.244$ ). (A) LC chromatogram and table of peak properties. %Area describes relative area under the curve of selected peaks and interpreted as an estimate of purity. (B) Mass spectrum



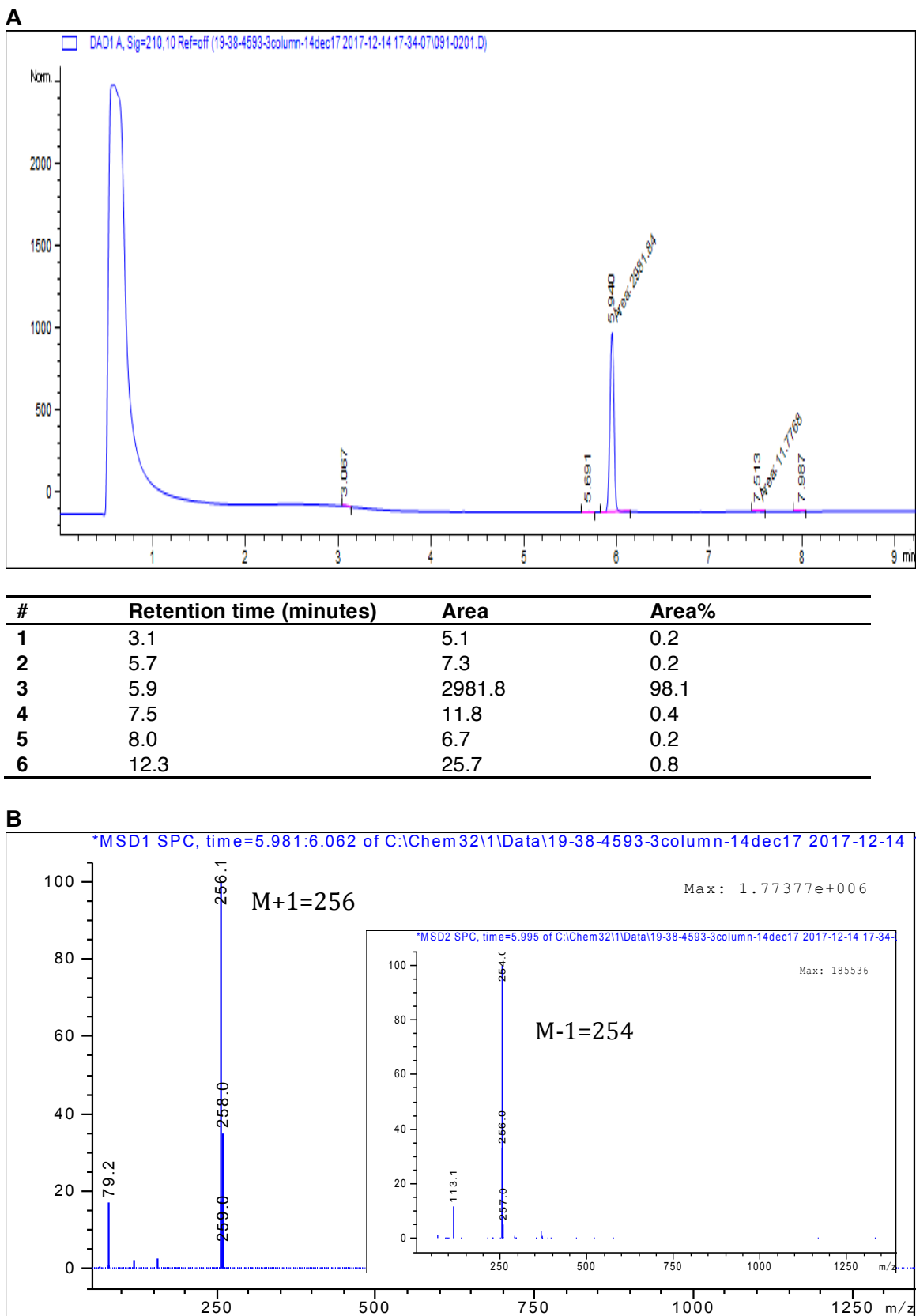
#	Retention time (minutes)	Area	Area%
2	5.1	5.2	0.1
3	5.7	3447	97.0
4	7.1	43.7	1.2
5	7.5	6.5	0.2
6	7.7	5.7	0.2
7	8.0	6.8	0.2
8	8.8	21.5	0.6



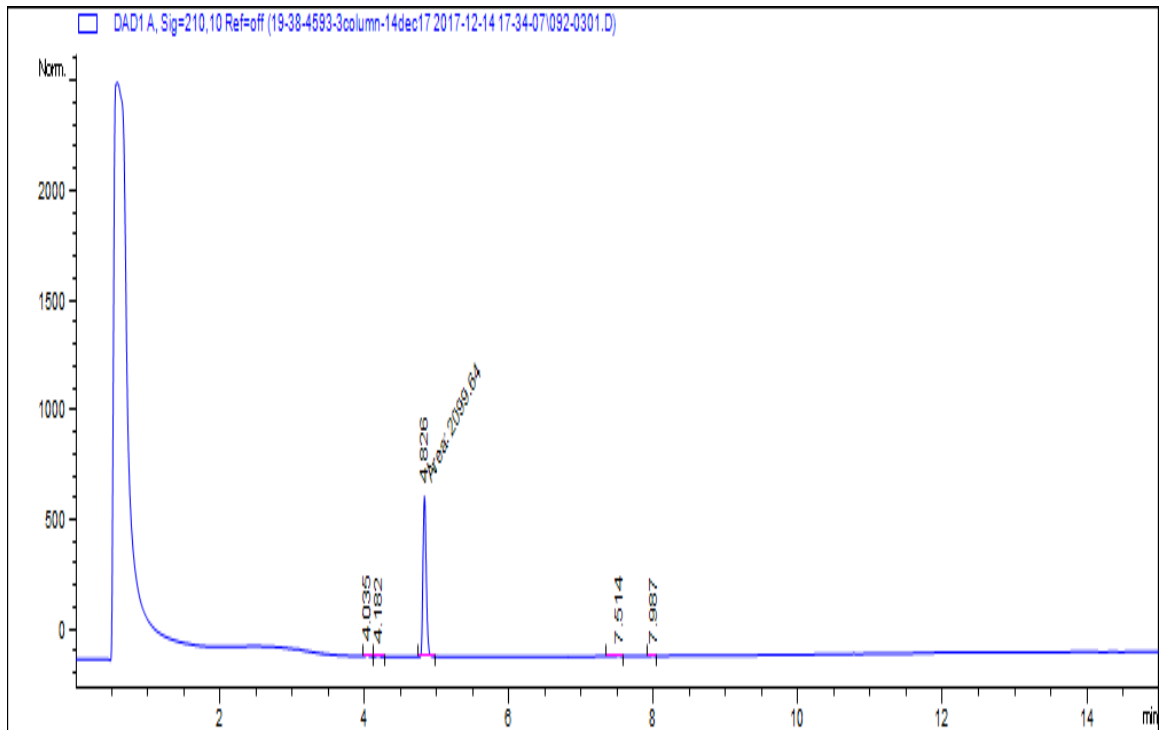
**Figure SI 10.** LC-MS chromatogram and mass spectrum for SM10. Molecular weight is 311.358 g/mol. (A) LC chromatogram and table of peak properties. %Area describes relative area under the curve of selected peaks and interpreted as an estimate of purity. (B) Mass spectrum



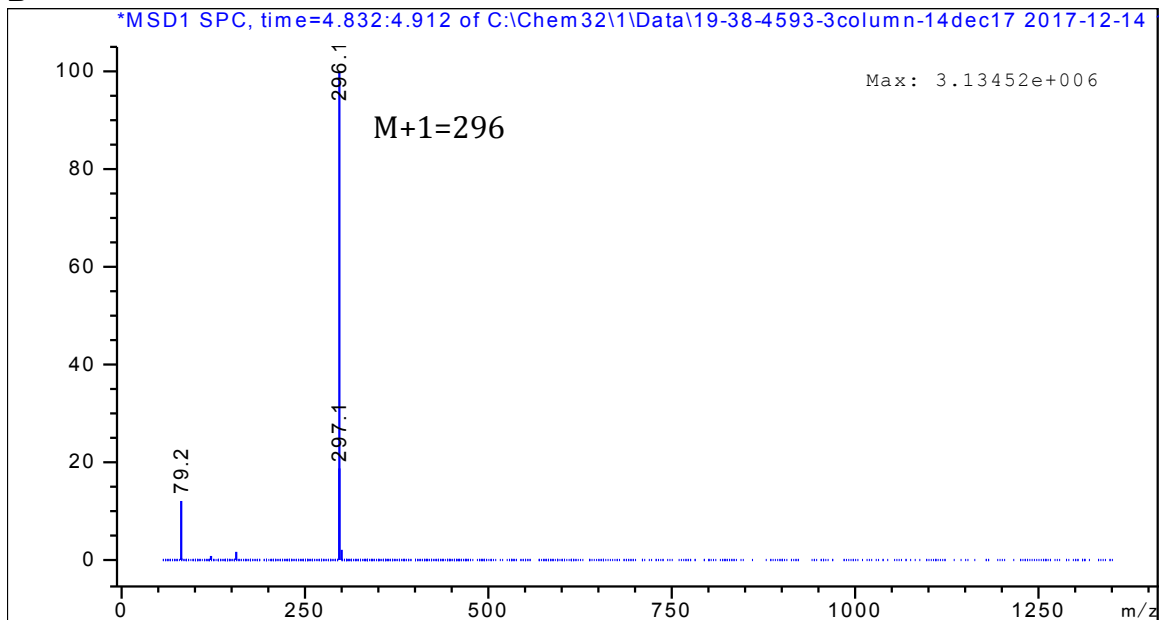
**Figure SI 11.** LC-MS chromatogram and mass spectrum for SM11. Molecular weight is 211.233 g/mol. (A) LC chromatogram and table of peak properties. %Area describes relative area under the curve of selected peaks and interpreted as an estimate of purity. (B) Mass spectrum



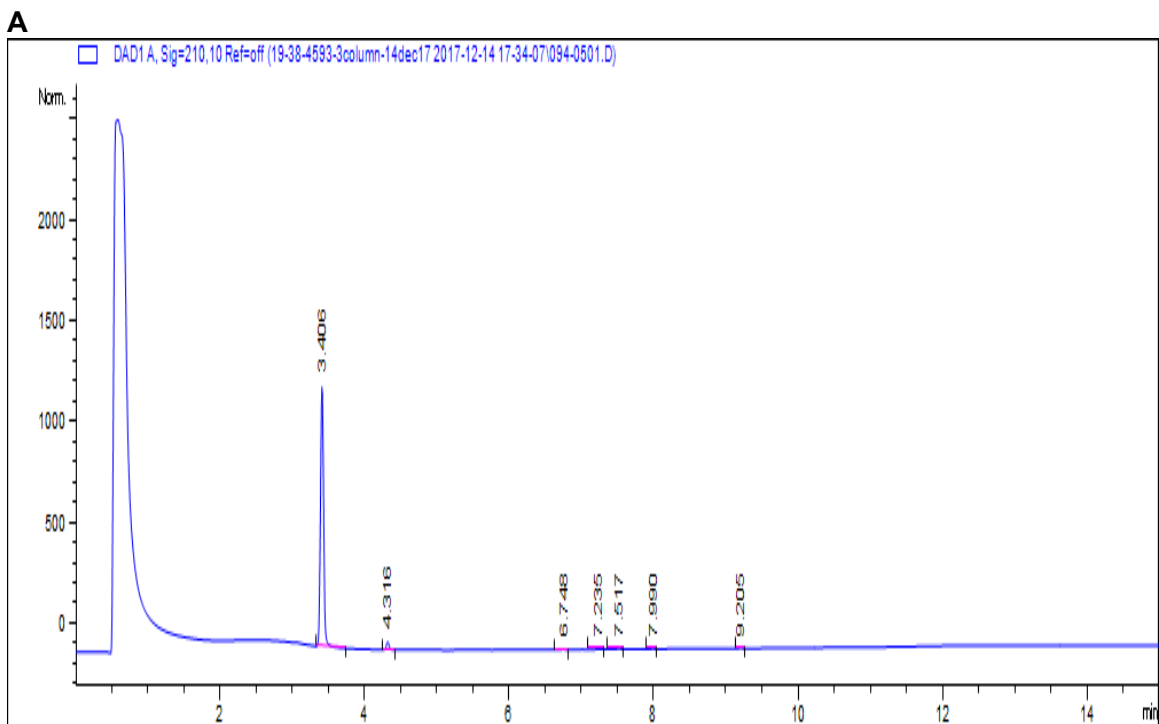
**Figure SI 12.** LC-MS chromatogram and mass spectrum for SM12. Molecular weight is 292.163 g/mol ( $m/z = 255$ ). (A) LC chromatogram and table of peak properties. %Area describes relative area under the curve of selected peaks and interpreted as an estimate of purity. (B) Mass spectrum

**A**

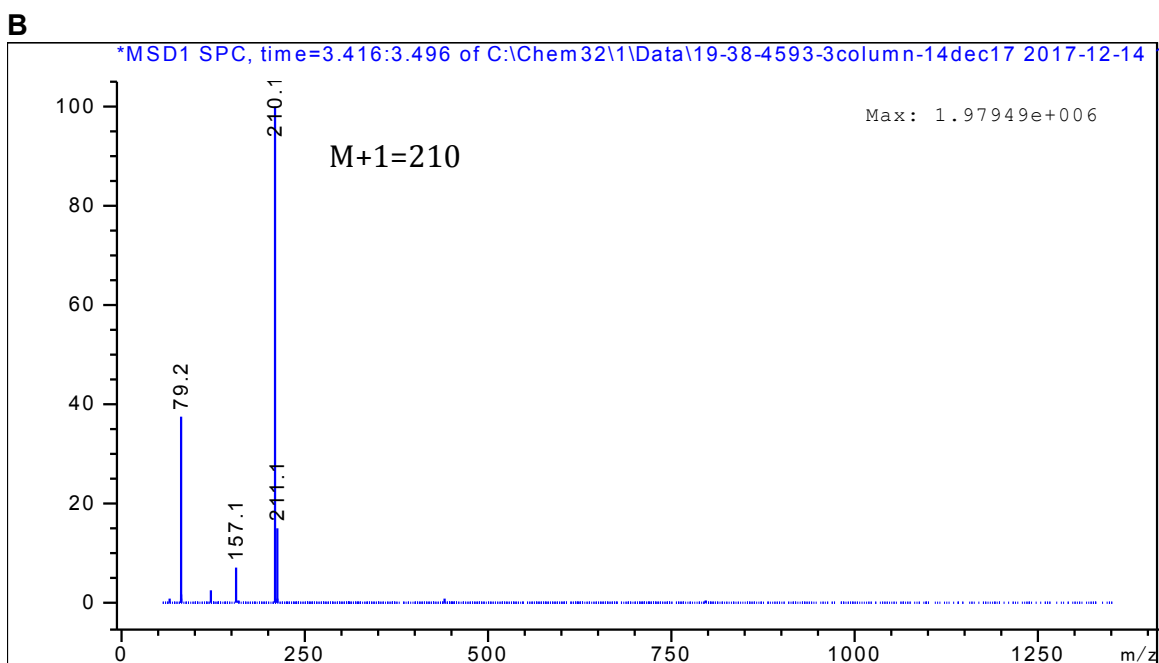
#	Retention time (minutes)	Area	Area%
1	4.0	18.1	0.8
2	4.2	15.4	0.7
3	4.8	2099.6	97.5
4	7.5	13.5	0.6
5	8.0	6.5	0.3

**B**

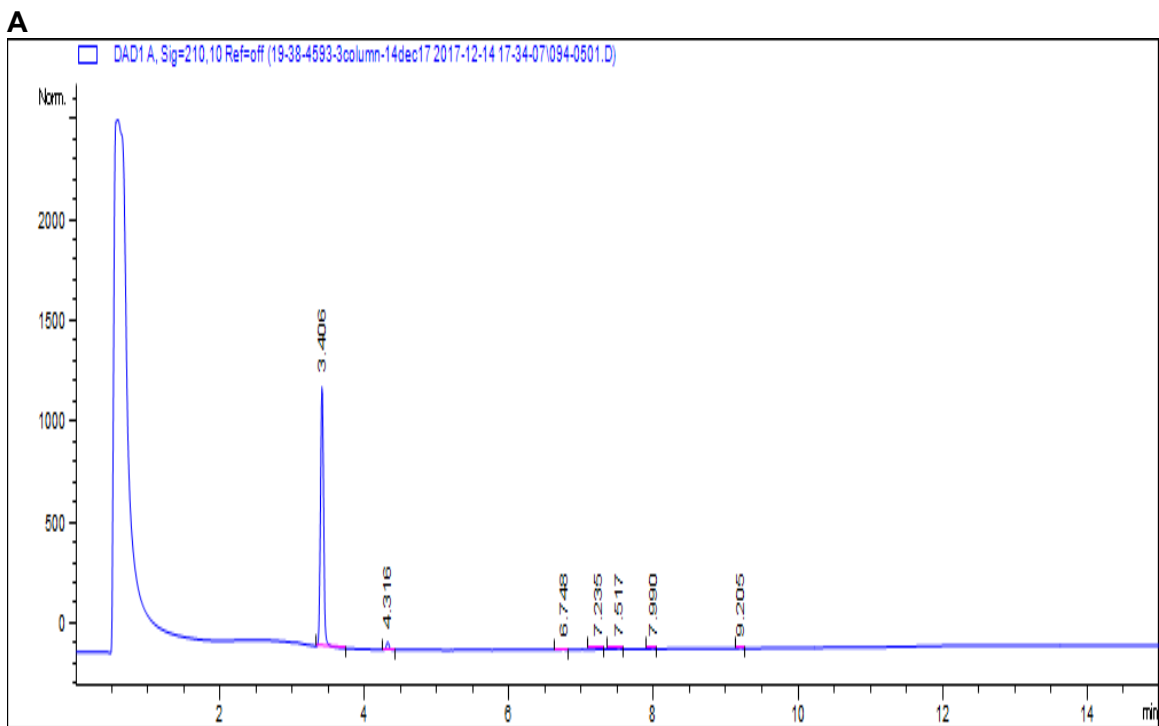
**Figure SI 13.** LC-MS chromatogram and mass spectrum for SM13. Molecular weight is 295.336 g/mol. (A) LC chromatogram and table of peak properties. %Area describes relative area under the curve of selected peaks and interpreted as an estimate of purity. (B) Mass spectrum



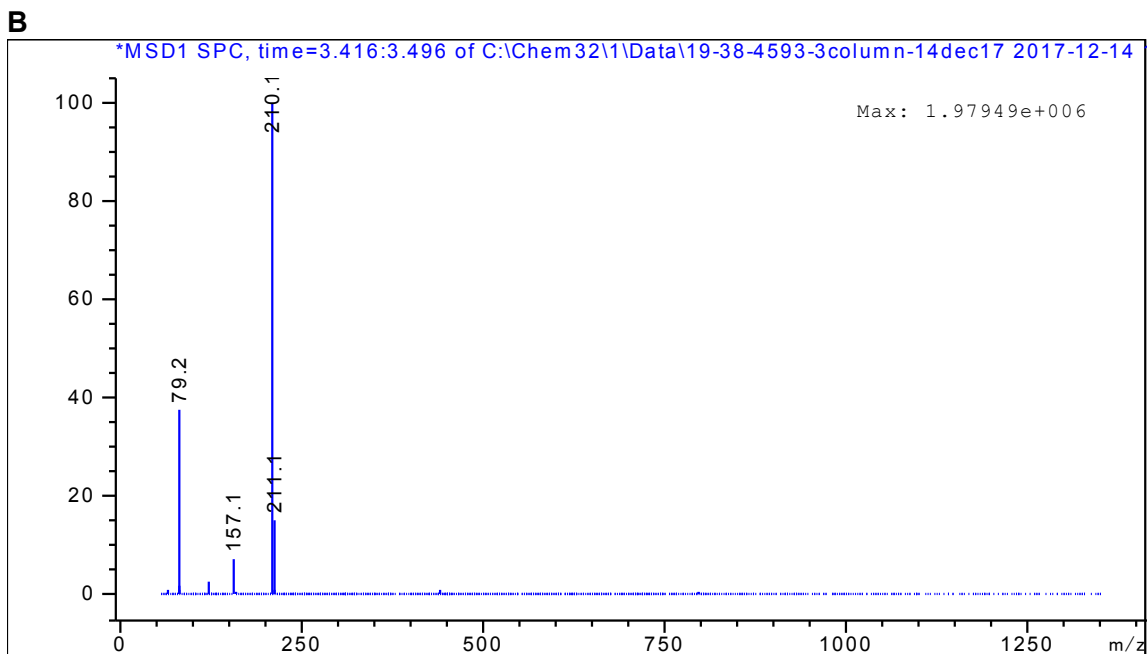
#	Retention time (minutes)	Area	Area%
1	3.4	3561.5	96.5
2	4.3	90.1	2.4
3	6.7	7.5	0.2
4	7.2	5.9	0.2
5	7.5	14.3	0.4
6	8.0	6.6	0.2
7	9.2	5.3	0.1



**Figure SI 14.** LC-MS chromatogram and mass spectrum for SM14. Molecular weight is 209.247 g/mol. (A) LC chromatogram and table of peak properties. %Area describes relative area under the curve of selected peaks and interpreted as an estimate of purity. (B) Mass spectrum

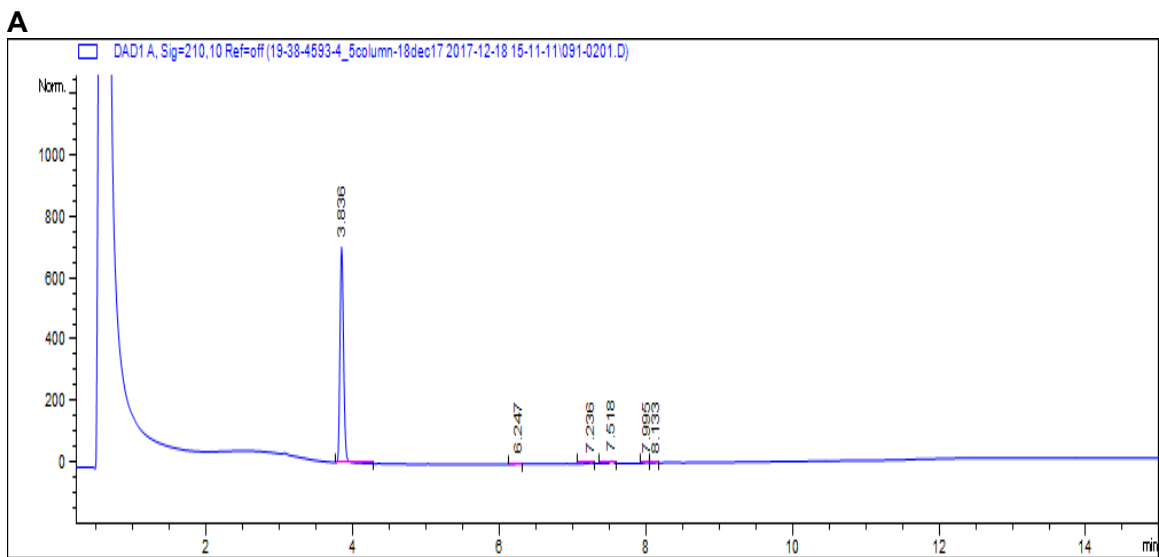


#	Retention time (minutes)	Area	Area%
1	4.4	2822.7	98.7
2	5.5	15.6	0.5
3	5.8	5.5	0.9
4	7.5	9.4	1.2
5	8.0	6.7	1.0

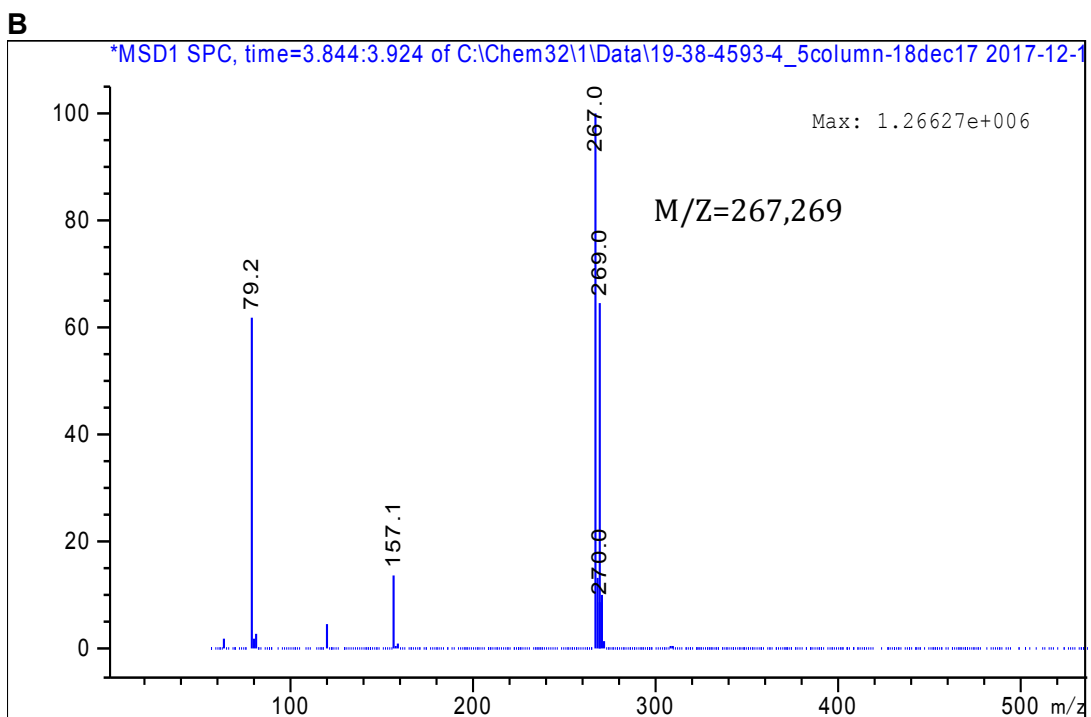


**Figure SI 15.** LC-MS chromatogram and mass spectrum for SM15. Molecular weight is 210.231 g/mol. (A) LC chromatogram and table of peak properties. %Area describes relative area under the curve of selected peaks and interpreted as an estimate of purity. (B) Mass spectrum

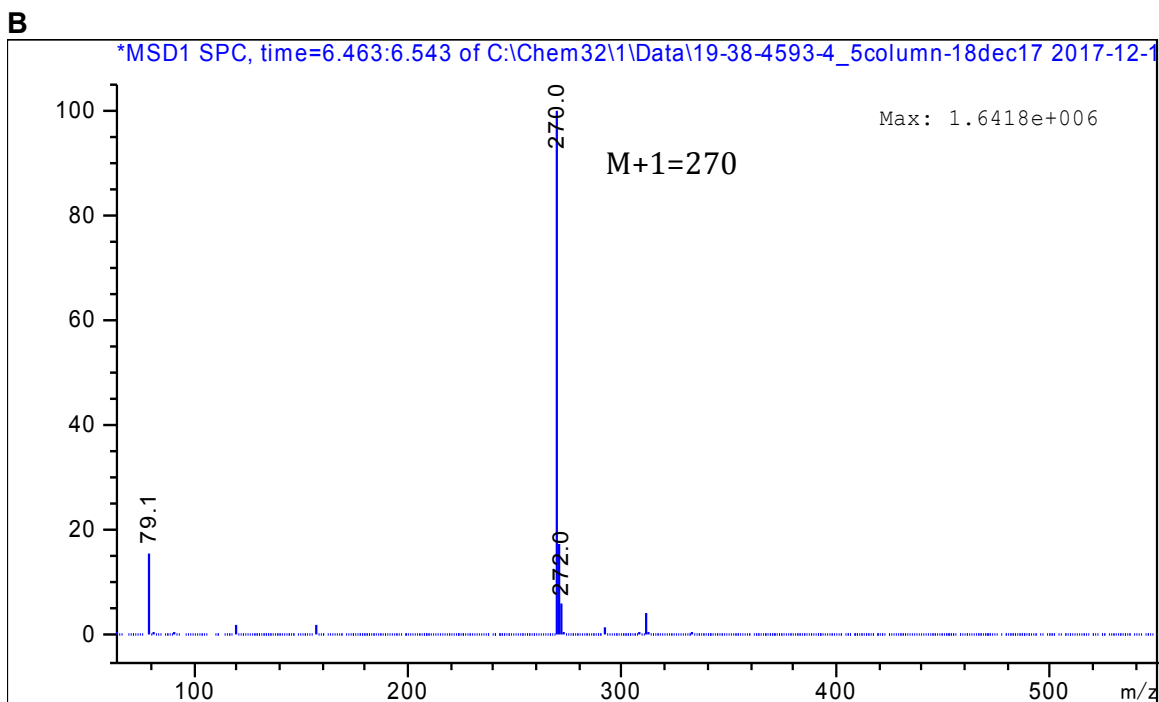
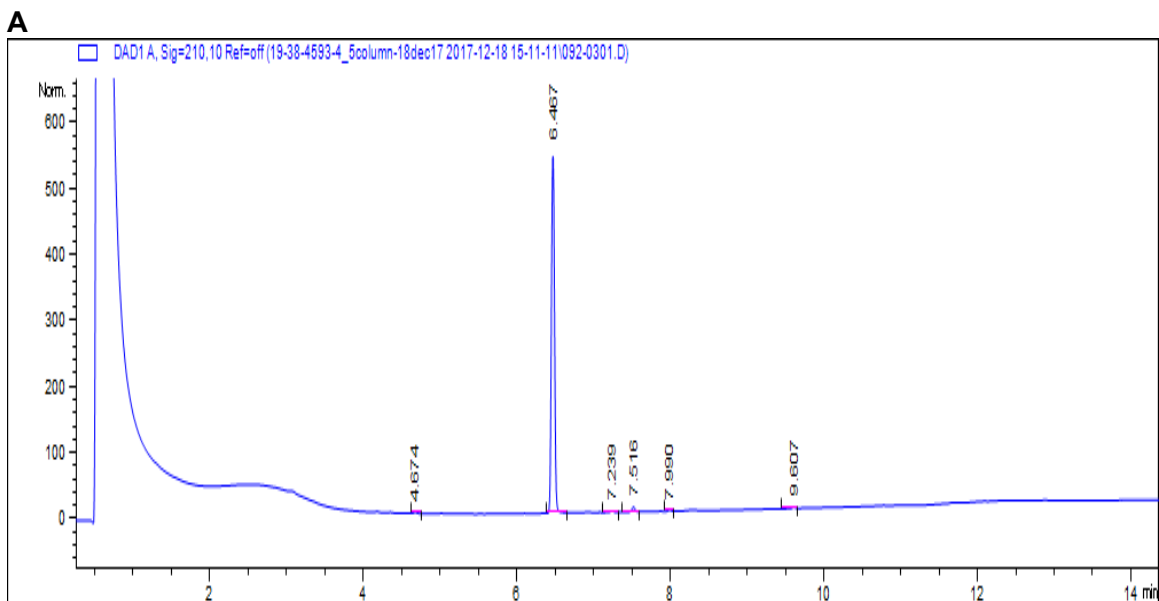




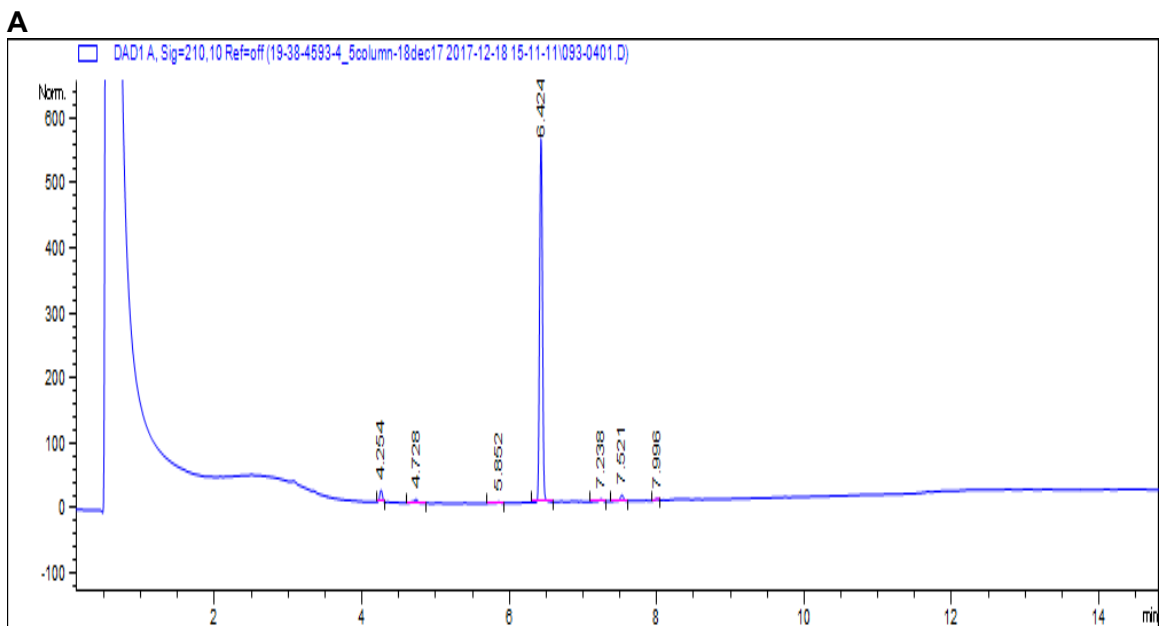
#	Retention time (minutes)	Area	Area%
1	3.8	2375.8	97.7
2	6.2	5.3	0.2
3	7.2	9.6	0.4
4	7.5	23.9	1.0
5	8.0	10.2	0.4
6	8.1	6.1	0.3



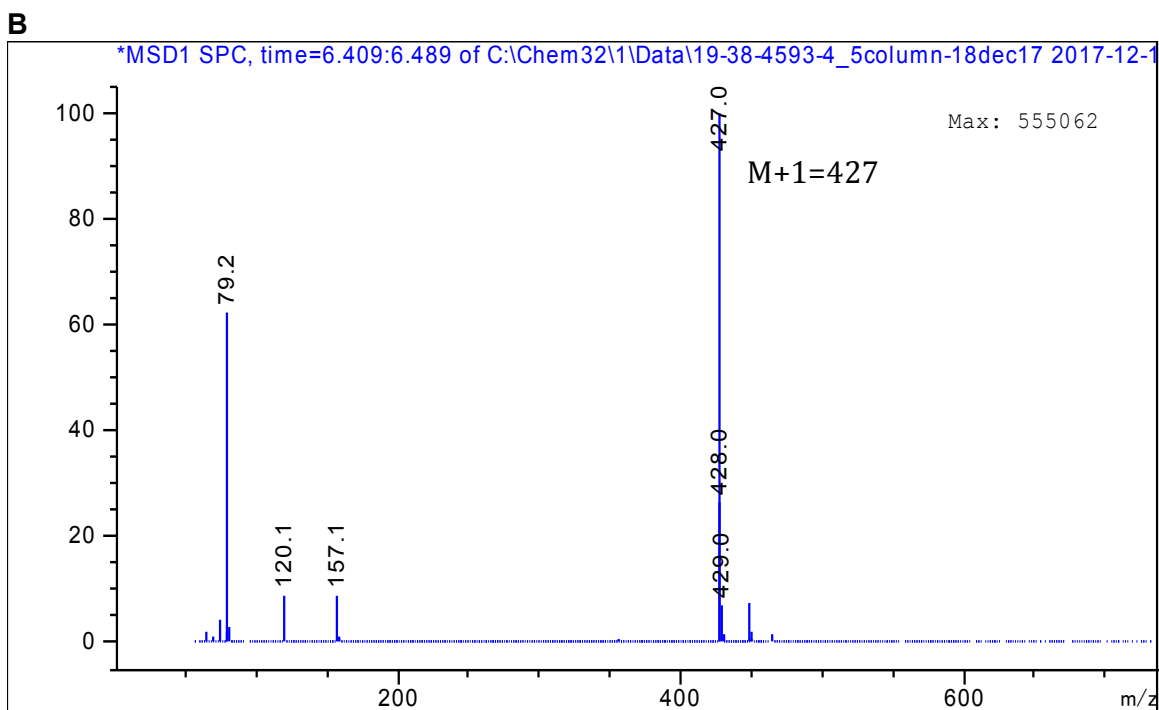
**Figure SI 16.** LC-MS chromatogram and mass spectrum for SM16. Molecular weight is 267.111 g/mol. (A) LC chromatogram and table of peak properties. %Area describes relative area under the curve of selected peaks and interpreted as an estimate of purity. (B) Mass spectrum



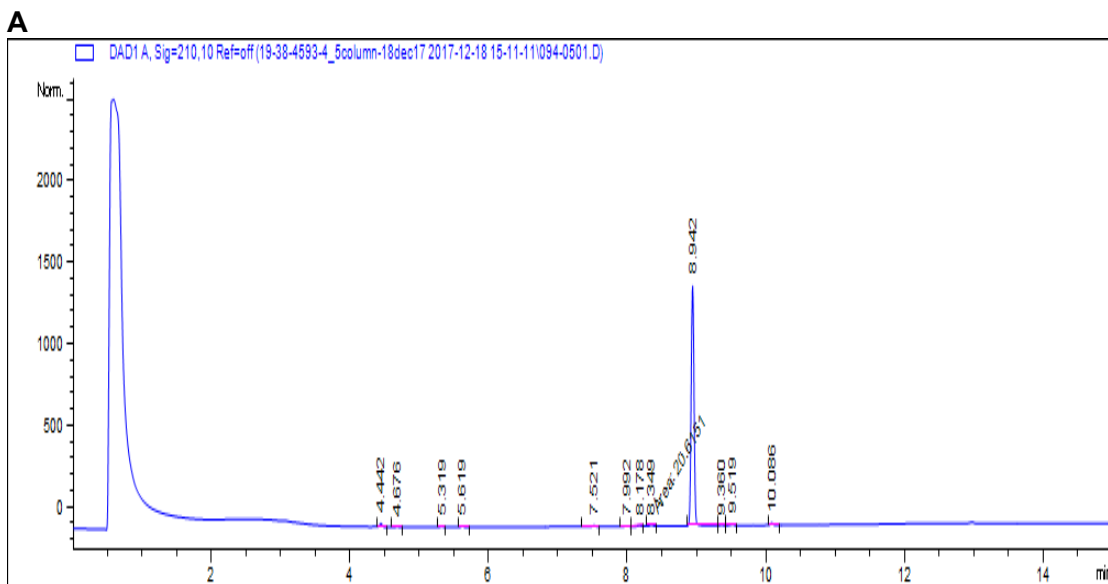
**Figure SI 17.** LC-MS chromatogram and mass spectrum for SM17. Molecular weight is 269.322 g/mol. (A) LC chromatogram and table of peak properties. %Area describes relative area under the curve of selected peaks and interpreted as an estimate of purity. (B) Mass spectrum



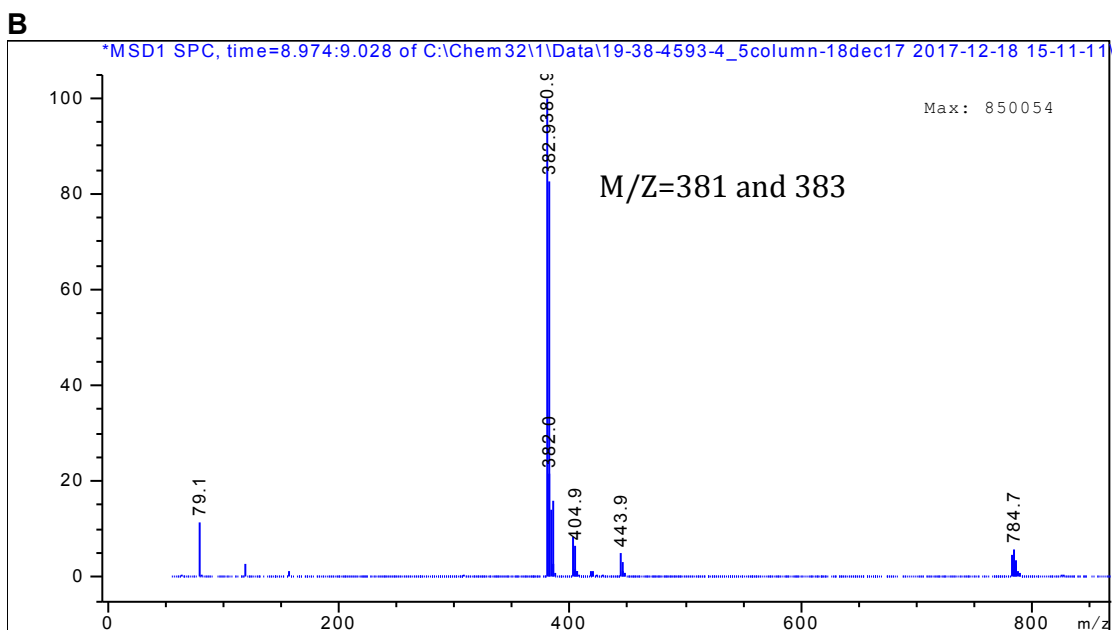
#	Retention time (minutes)	Area	Area%
1	4.3	47.8	2.9
2	4.7	22.5	1.4
3	5.9	5.7	0.3
4	6.4	1538.3	92.6
5	7.2	10.7	0.6
6	7.5	28.5	1.7
7	8.0	7.8	0.5



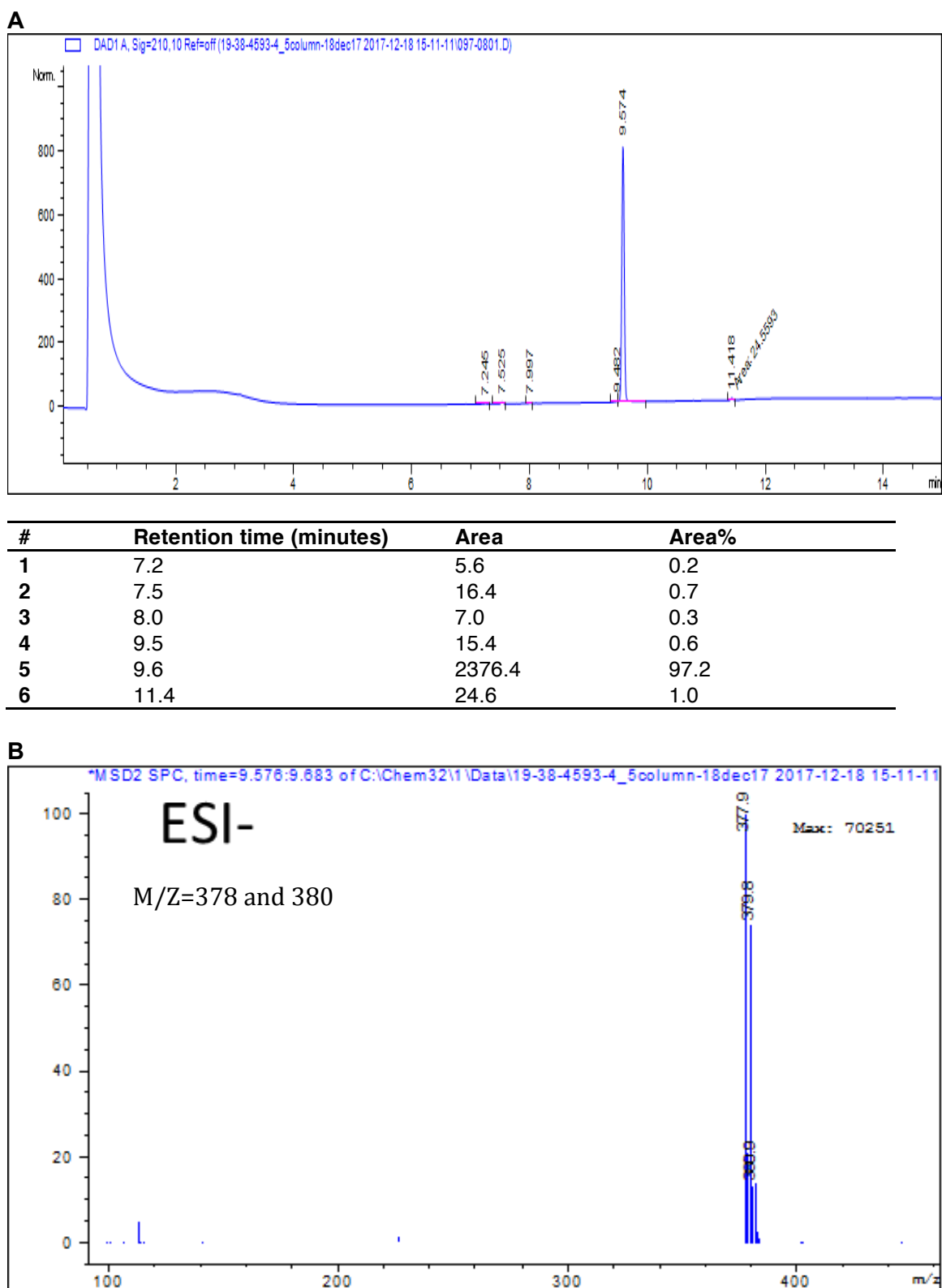
**Figure SI 18.** LC-MS chromatogram and mass spectrum for SM18. Molecular weight is 426.439 g/mol. (A) LC chromatogram and table of peak properties. %Area describes relative area under the curve of selected peaks and interpreted as an estimate of purity. (B) Mass spectrum



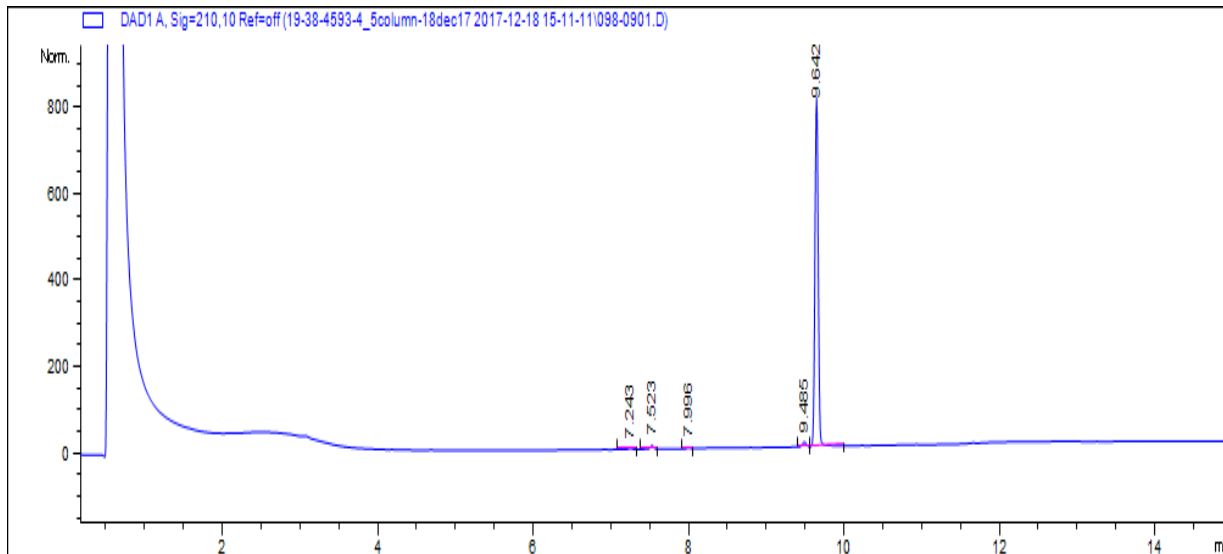
#	Retention time (minutes)	Area	Area%
1	4.4	51.6	1.3
2	4.7	5.7	0.1
3	5.3	14.3	0.3
4	5.6	17.8	0.4
5	7.5	17.4	0.4
6	8.0	8.2	0.2
7	8.2	14.1	0.3
8	8.3	20.6	0.5
9	8.9	3893.8	94.5
10	9.4	15.4	0.4
11	9.5	26.1	0.6
12	10.1	37.1	0.9



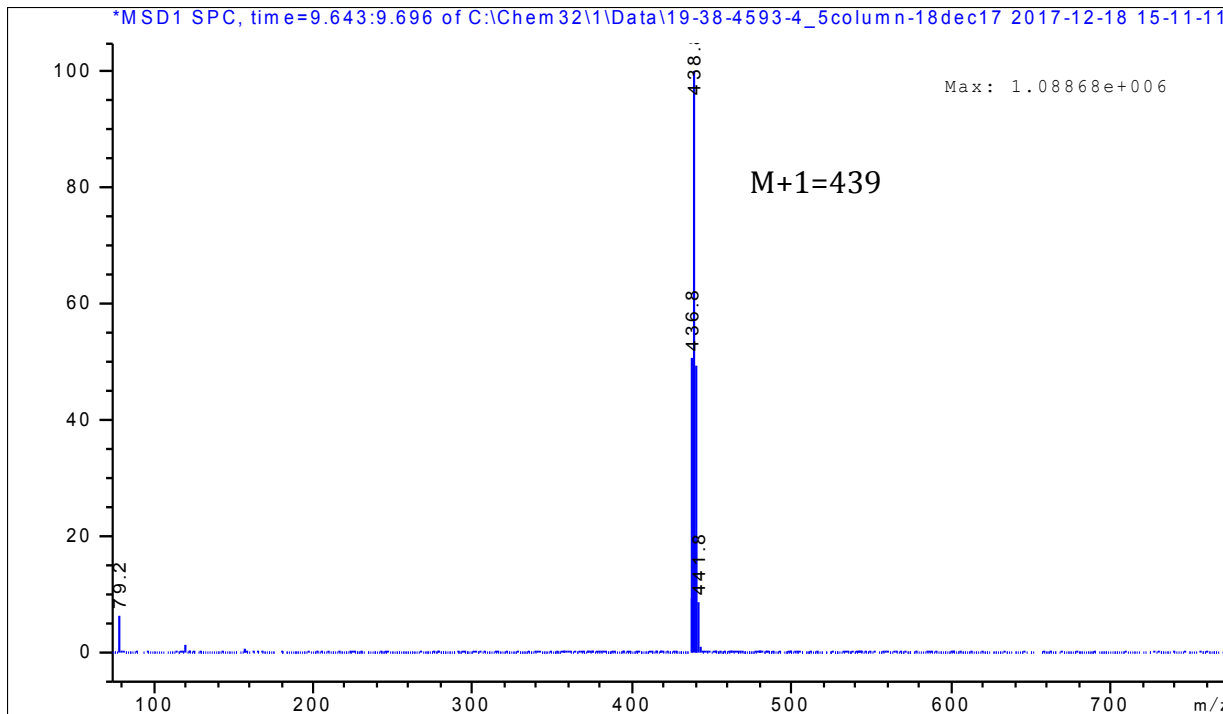
**Figure SI 19.** LC-MS chromatogram and mass spectrum for SM19. Molecular weight is 381.276 g/mol. (A) LC chromatogram and table of peak properties. %Area describes relative area under the curve of selected peaks and interpreted as an estimate of purity. (B) Mass spectrum



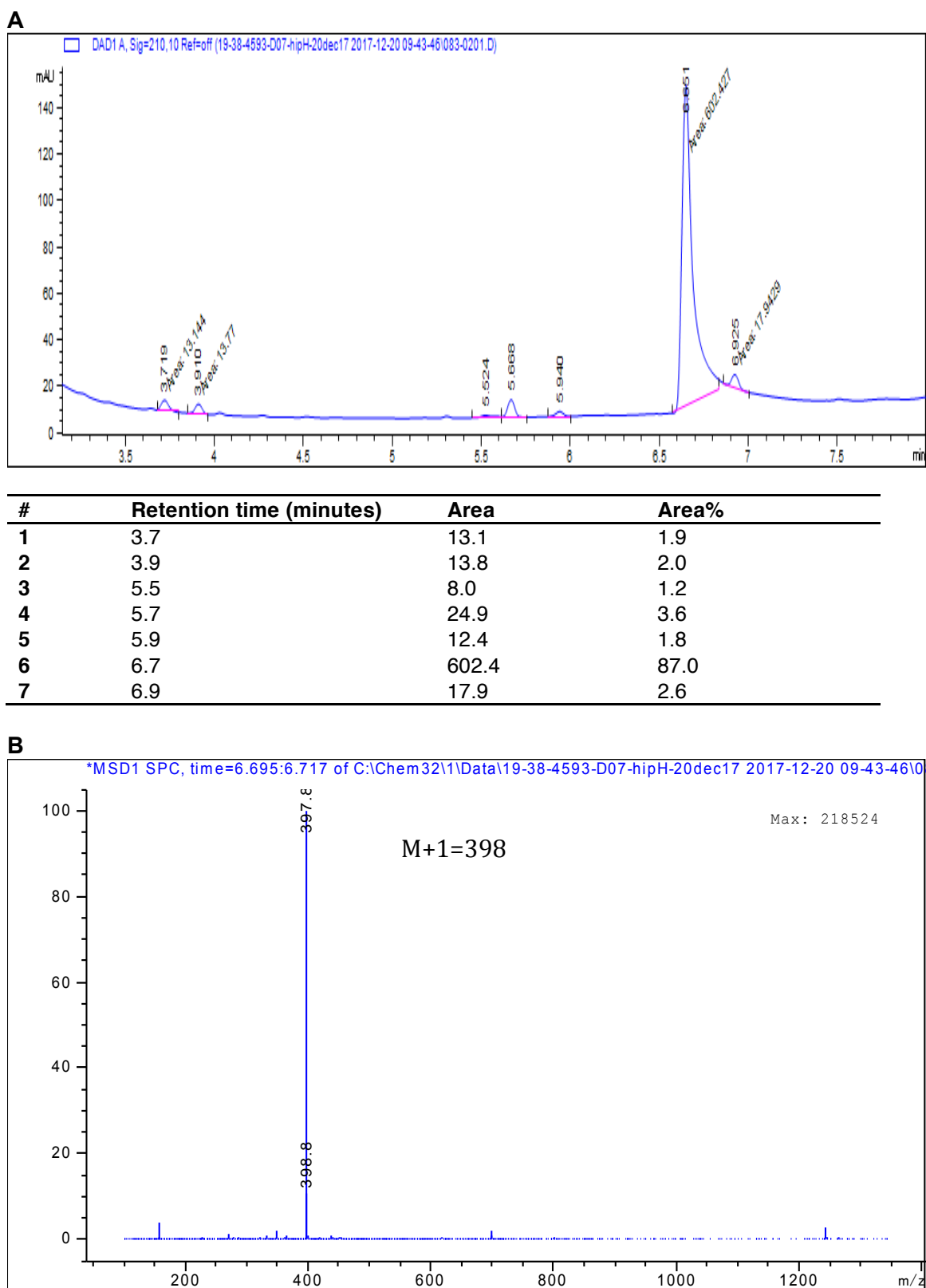
**Figure SI 20.** LC-MS chromatogram mass spectrum for SM20. Molecular weight is 380.245 g/mol. (A) LC chromatogram and table of peak properties. %Area describes relative area under the curve of selected peaks and interpreted as an estimate of purity. (B) Mass spectrum

**A**

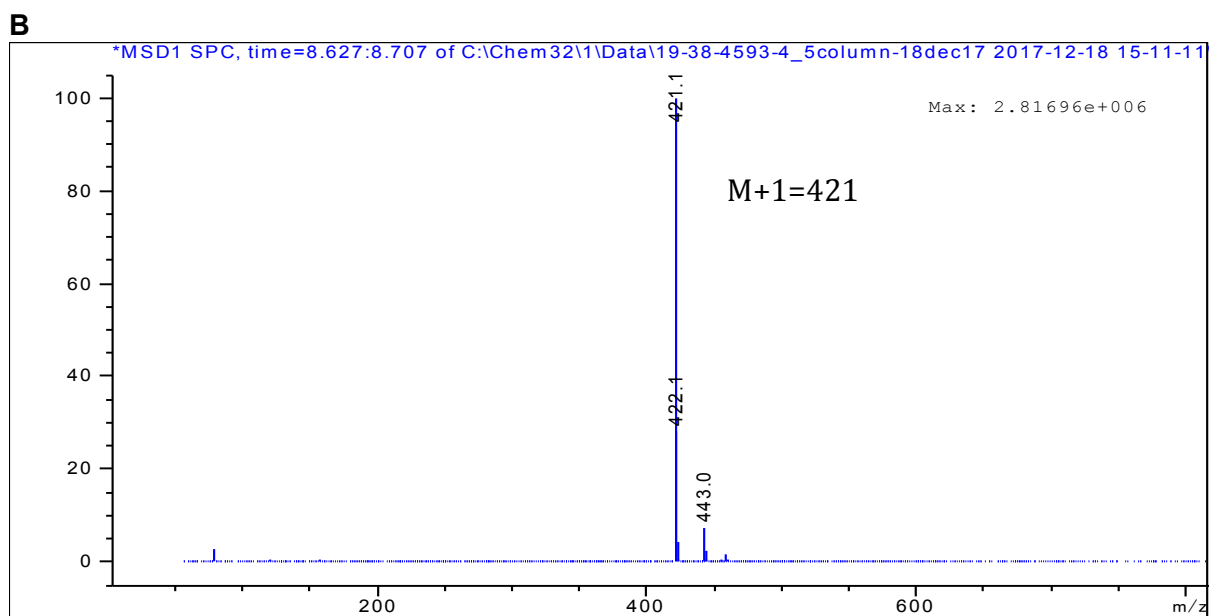
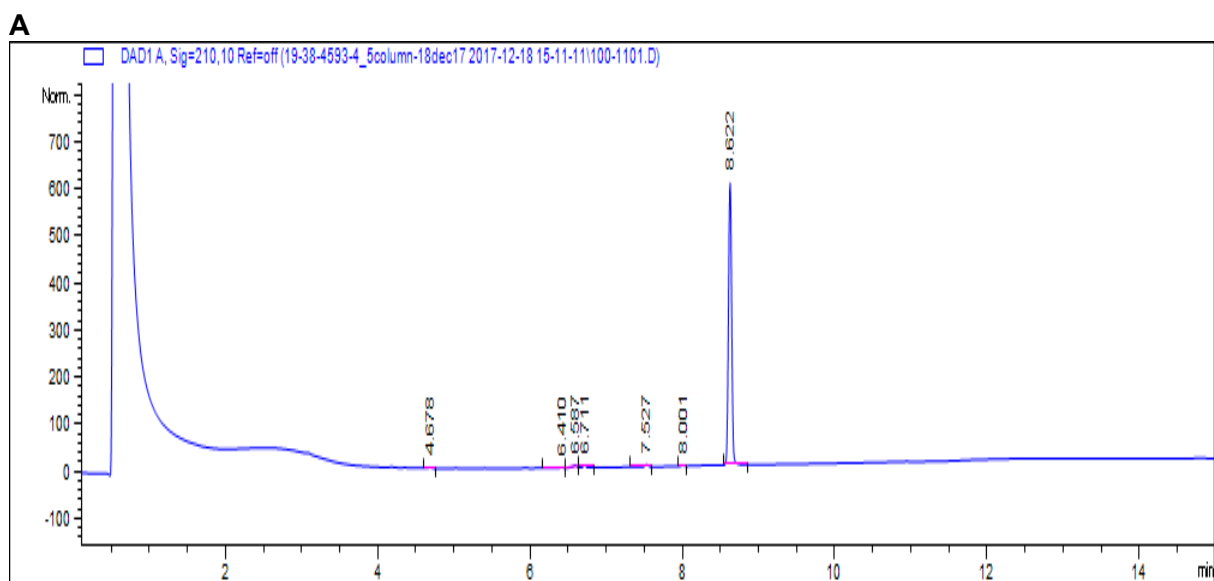
#	Retention time (minutes)	Area	Area%
1	7.2	9.1	0.4
2	7.5	23.6	1.0
3	8.0	6.4	0.2
4	9.5	39.9	1.6
5	9.6	2381.2	96.8

**B**

**Figure SI 21.** LC-MS chromatogram and mass spectrum for SM21. Molecular weight is 438.092 g/mol. (A) LC chromatogram and table of peak properties. %Area describes relative area under the curve of selected peaks and interpreted as an estimate of purity. (B) Mass spectrum

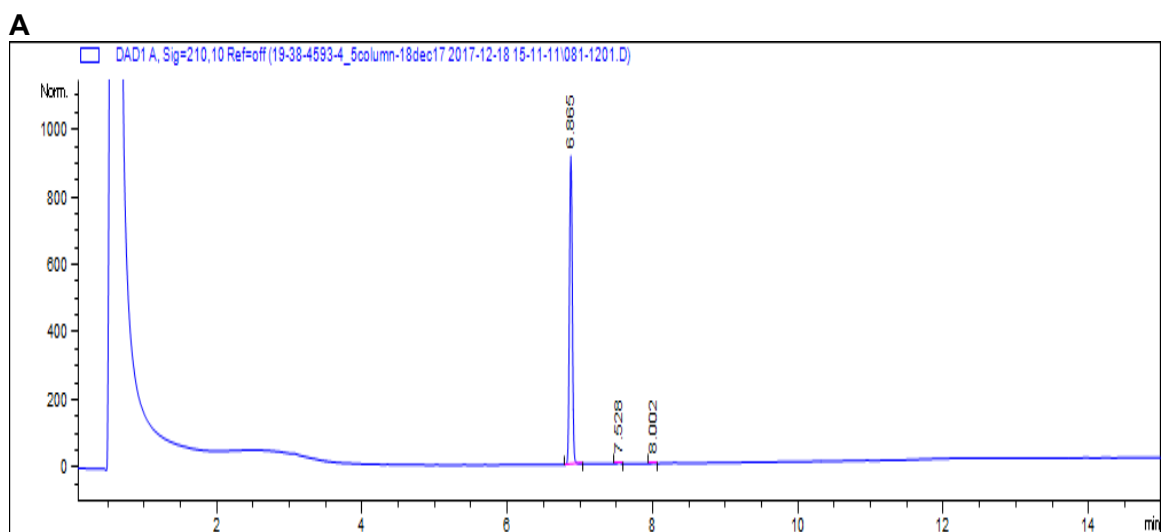


**Figure SI 22.** LC-MS chromatogram and mass spectrum for SM22. Molecular weight is 396.951 g/mol. (A) LC chromatogram and table of peak properties. %Area describes relative area under the curve of selected peaks and interpreted as an estimate of purity. (B) Mass spectrum

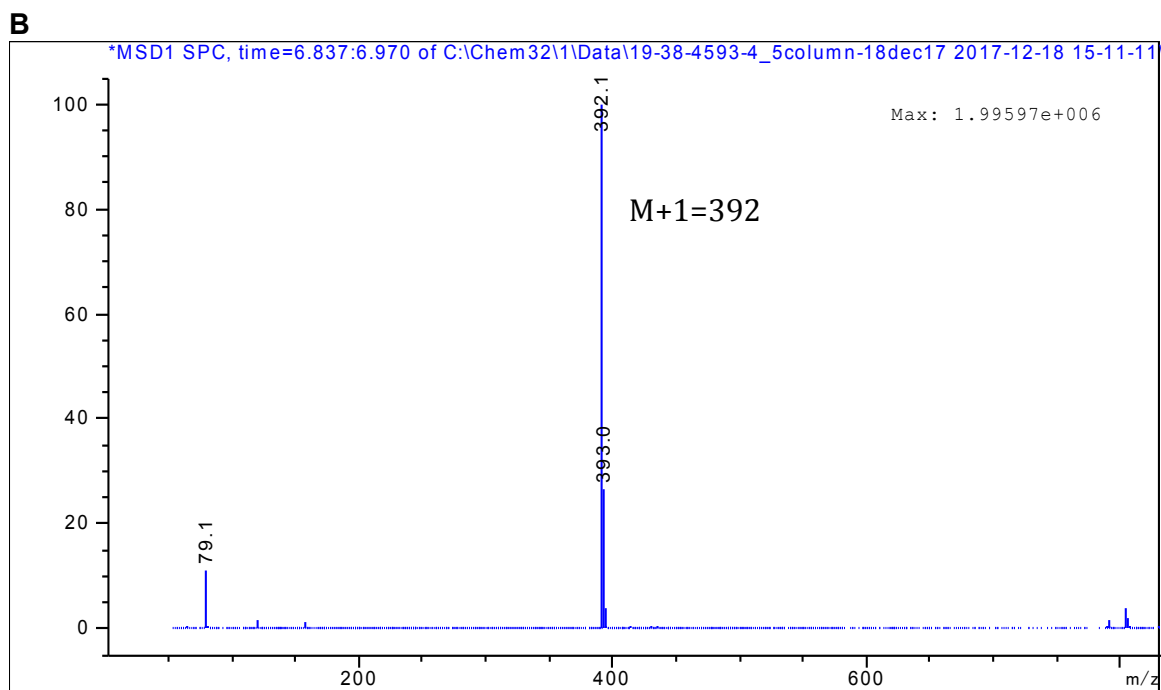


**Figure SI 23.** LC-MS chromatogram and mass spectrum for SM23. Molecular weight is 420.461 g/mol. (A) LC chromatogram and table of peak properties. %Area describes relative area under the curve of selected peaks and interpreted as an estimate of purity. (B) Mass spectrum



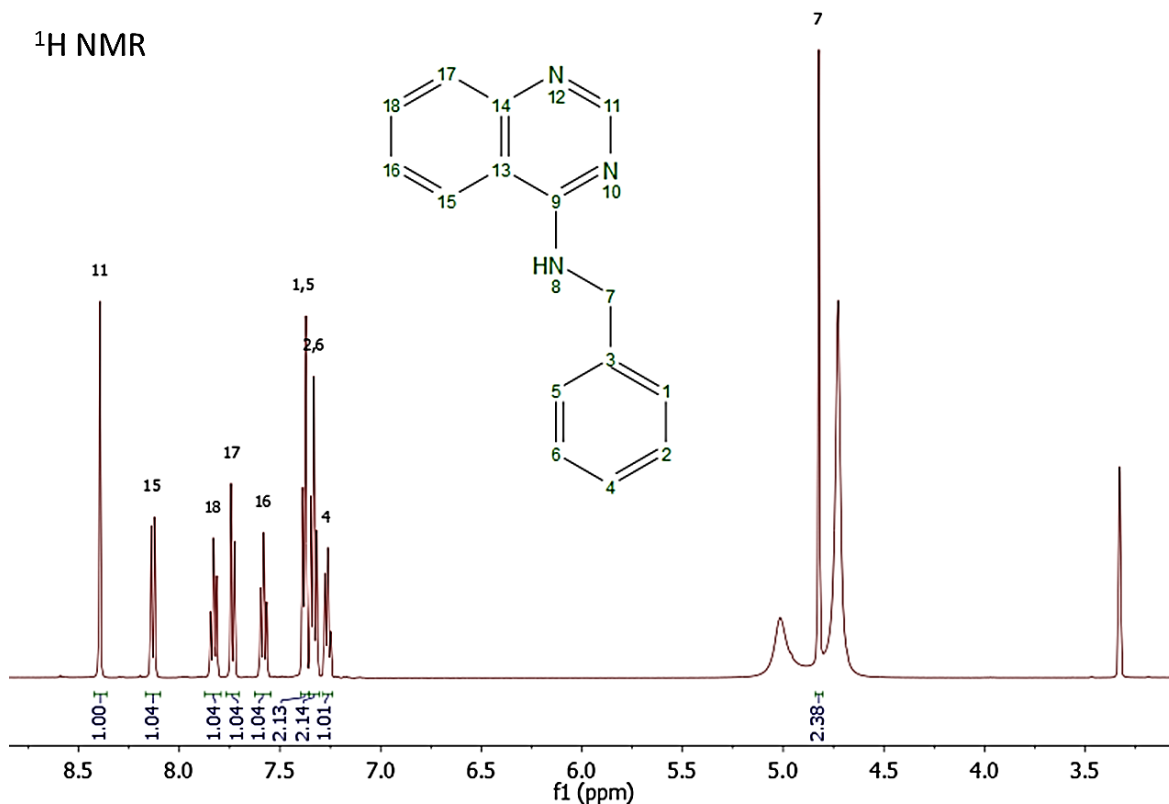


#	Retention time (minutes)	Area	Area%
1	6.9	2511.1	99.3
2	7.5	11.0	0.4
3	8.0	5.9	0.2



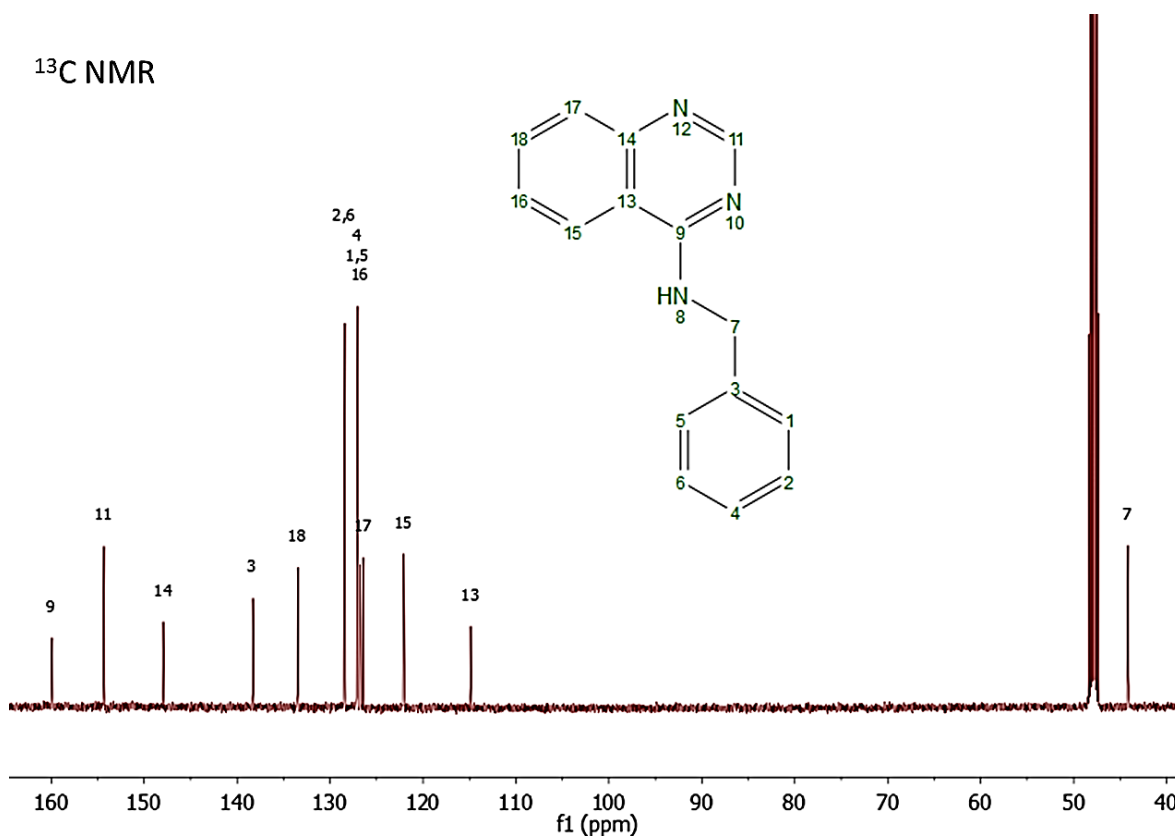
**Figure SI 24.** LC-MS chromatogram and mass spectrum for SM24. Molecular weight is 391.42 g/mol. (A) LC chromatogram and table of peak properties. %Area describes relative area under the curve of selected peaks and interpreted as an estimate of purity. (B) Mass spectrum

# <sup>1</sup>H NMR

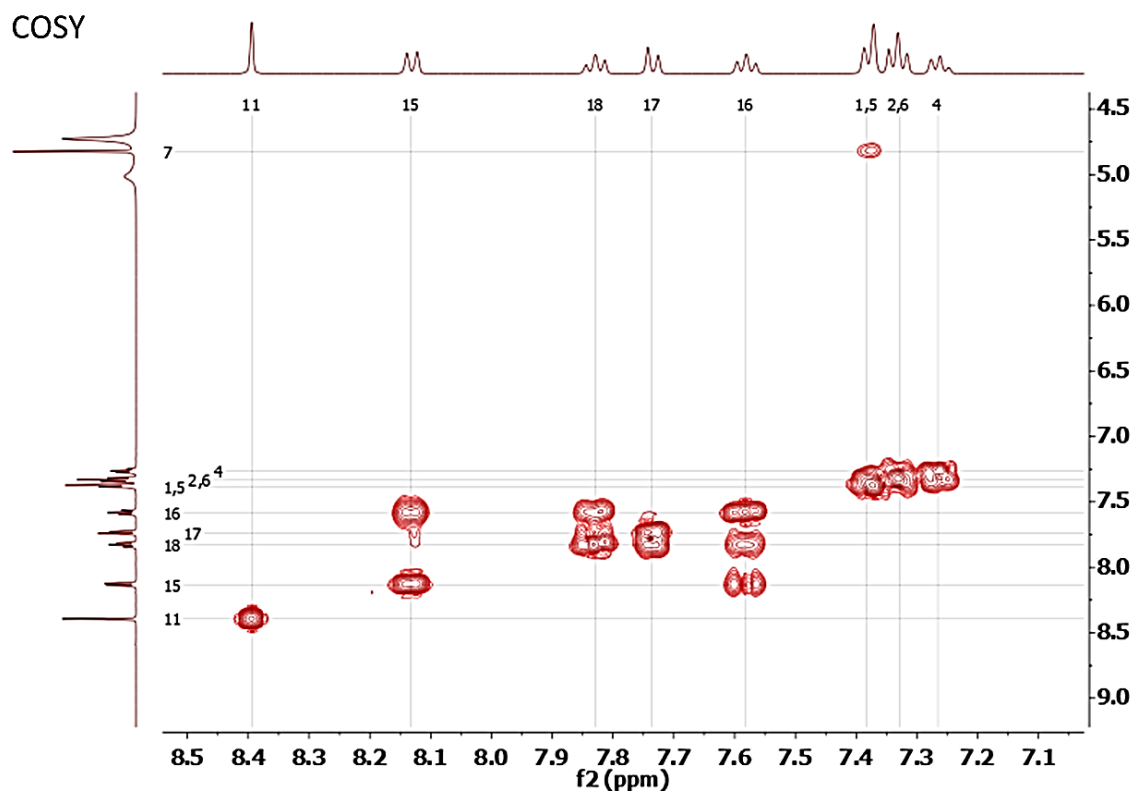


**Figure SI 25.** <sup>1</sup>H NMR spectrum (8 scans) of SM07 (5.8mg) showing resonances assigned to the corresponding protons. The spectrum was acquired on a Bruker 500 MHz spectrometer equipped with a 5 mm TCI CryoProbe<sup>TM</sup> prodigy at 298 K in 0.6 ml water-*d*<sub>2</sub>/methanol-*d*<sub>4</sub> (1:2).

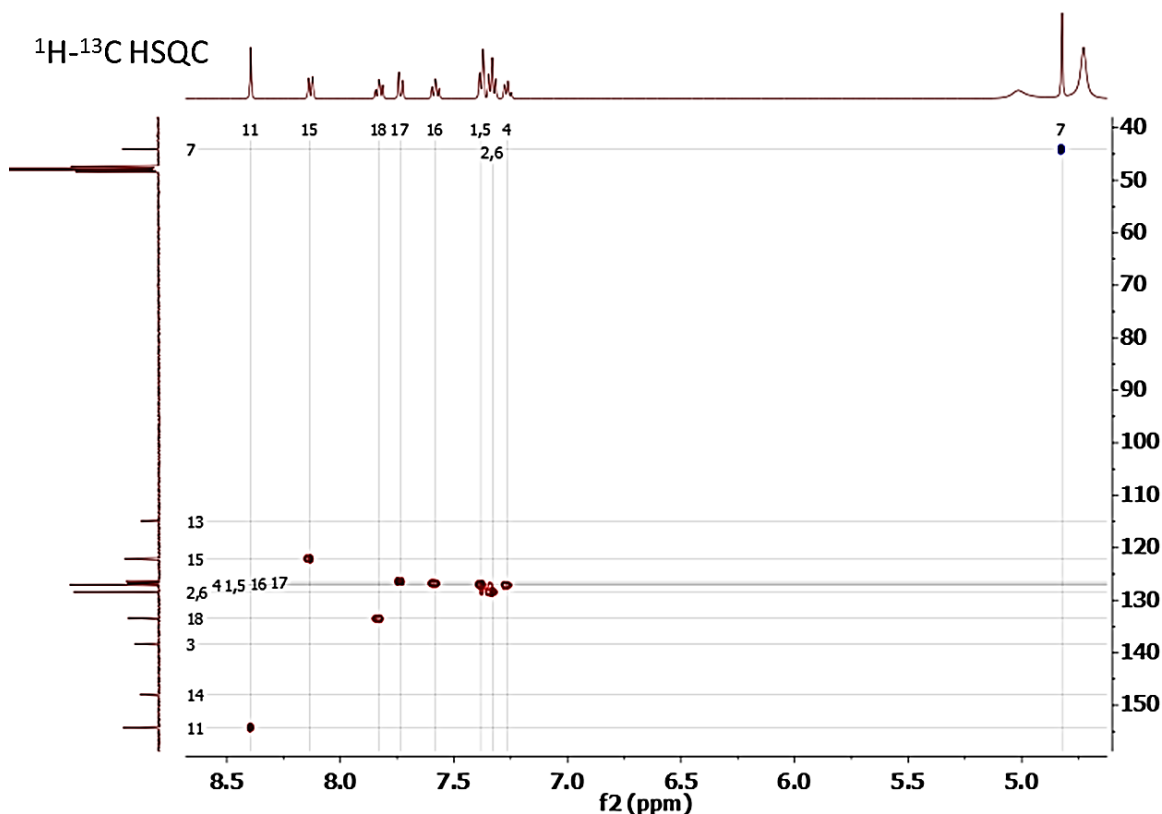
# <sup>13</sup>C NMR



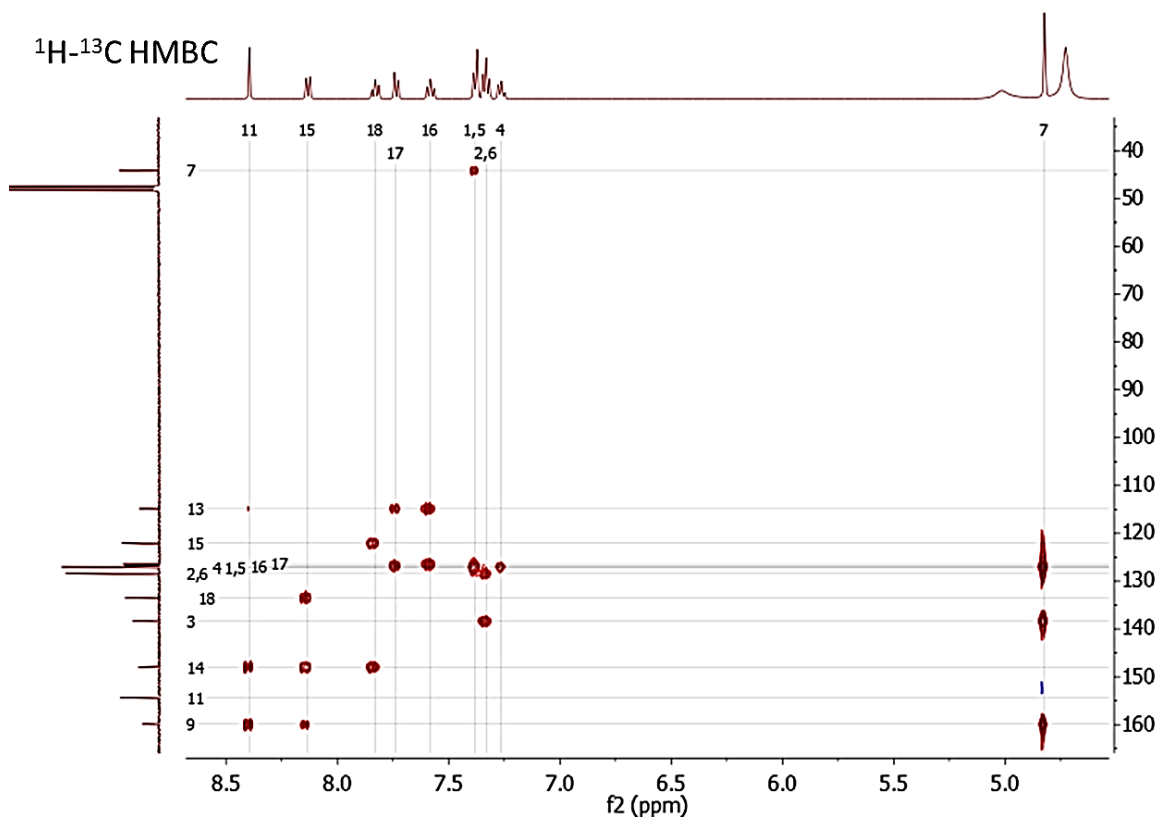
**Figure SI 26.** <sup>13</sup>C NMR spectrum (749 scans) of SM07 (5.8 mg) showing resonances assigned to the corresponding carbon nuclei. The spectrum was acquired on a Bruker 500 MHz spectrometer equipped with a 5 mm TCI CryoProbe<sup>TM</sup> prodigy at 298 K in 0.6 ml water-*d*<sub>2</sub>/methanol-*d*<sub>4</sub> (1:2).



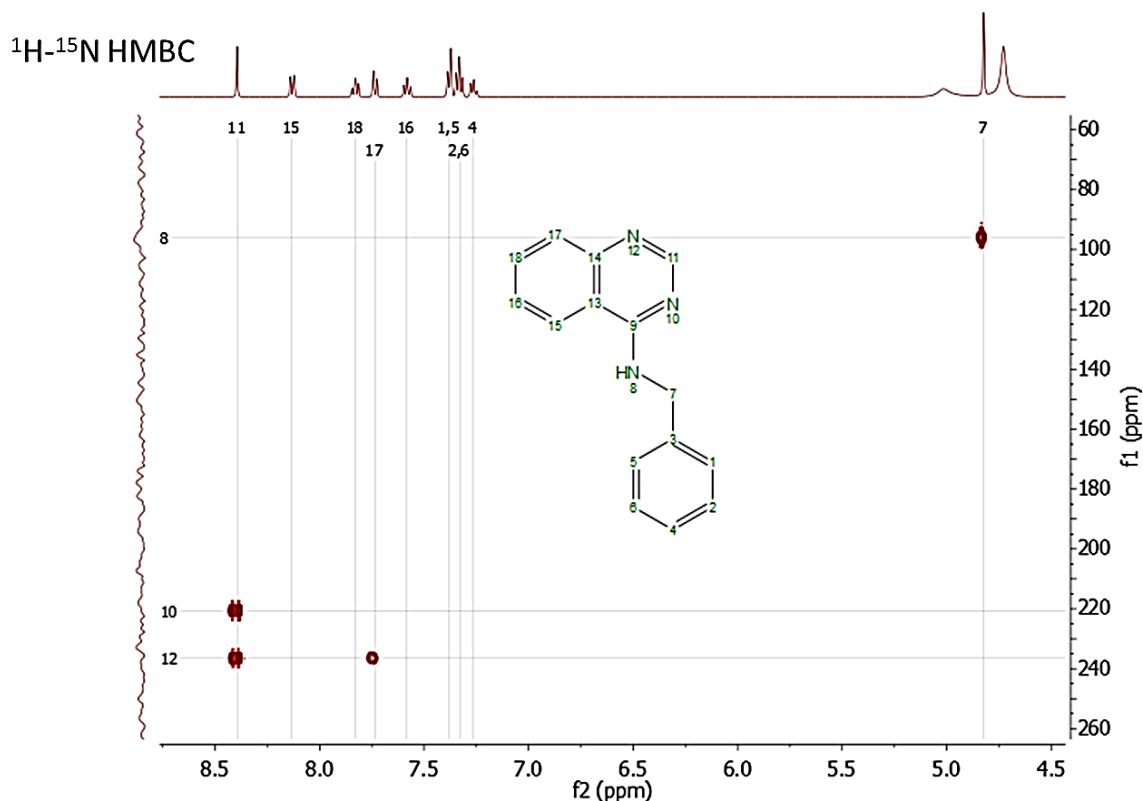
**Figure SI 27.** A 4 scan Correlated Spectroscopy (COSY) spectrum of SM07 (5.8 mg) acquired on a Bruker 500 MHz spectrometer equipped with a 5 mm TCI CryoProbe™ prodigy at 298 K in 0.6 ml water- $d_2$ /methanol- $d_4$  (1:2). Cross-peaks (off-diagonal peaks) corresponds to coupled adjacent protons.



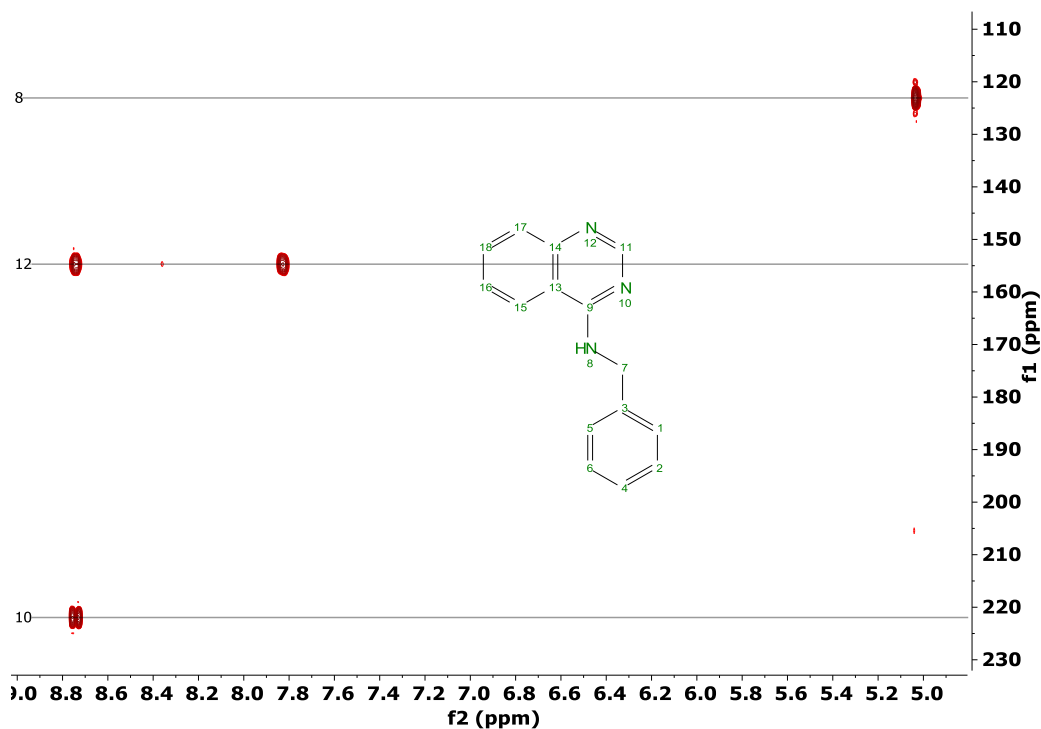
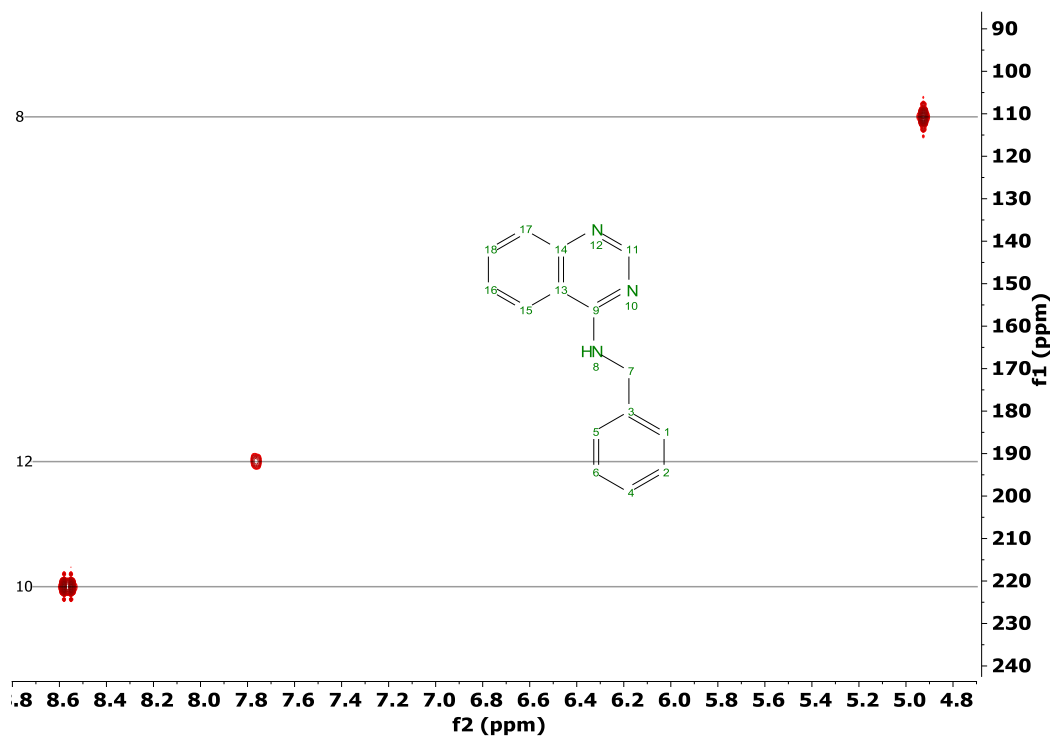
**Figure SI 28.** A 2 scan  $^1\text{H}$ - $^{13}\text{C}$  HSQC (heteronuclear single quantum correlation) spectrum of SM07 (5.8 mg) acquired on a Bruker 500 MHz spectrometer equipped with a 5 mm TCI CryoProbe™ prodigy at 298 K in 0.6 ml water- $d_2$ /methanol- $d_4$  (1:2). Each peak correlates a carbon to its attached proton(s).

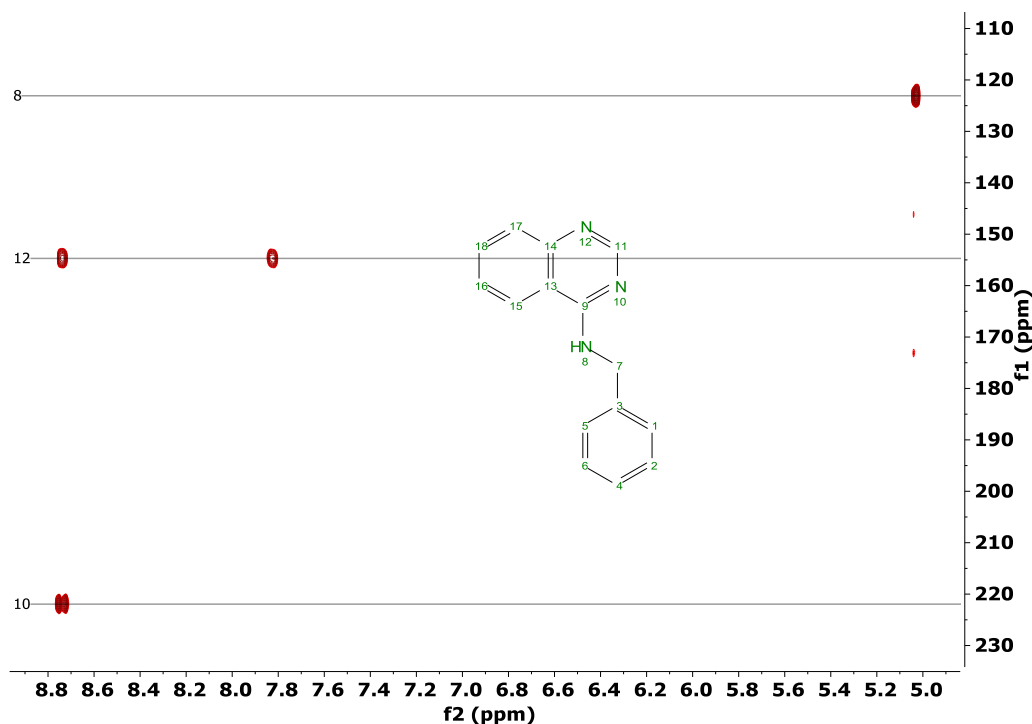


**Figure SI 29** A 4 scan  $^1\text{H}$ - $^{13}\text{C}$  HMBC (heteronuclear multiple bond correlation) spectrum of SM07 (5.8 mg) acquired on a Bruker 500 MHz spectrometer equipped with a 5 mm TCI CryoProbe<sup>TM</sup> prodigy at 298 K in 0.6 ml water- $d_2$ /methanol- $d_4$  (1:2). Peaks corresponds to coupling between the proton of interest to neighboring carbons, which are 2 – 4 bonds away.

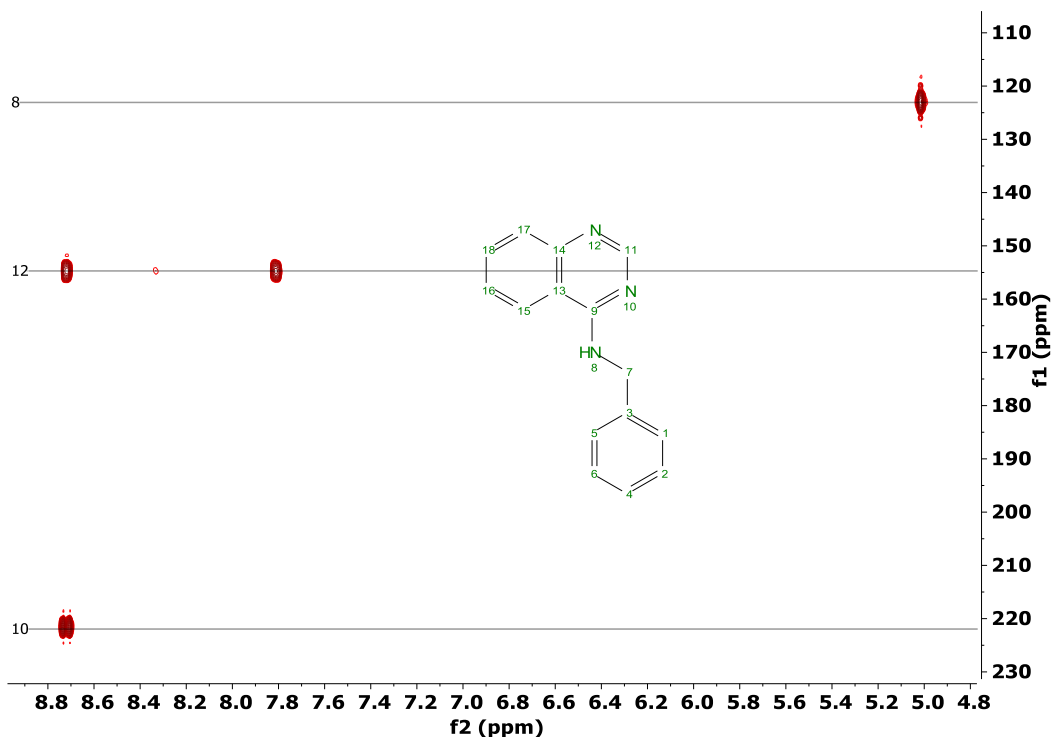


**Figure SI 30.** A 64 scan  $^1\text{H}$ - $^{15}\text{N}$  HMBC (heteronuclear multiple bond correlation) spectrum of SM07 (5.8 mg) acquired on a Bruker 500 MHz spectrometer equipped with a 5 mm TCI CryoProbe<sup>TM</sup> prodigy at 298 K in 0.6 ml water- $d_2$ /methanol- $d_4$  (1:2). The  $^{15}\text{N}$  chemical shifts have been assigned by their correlation to near-by protons. Peaks corresponds to coupling between the proton of interest to neighboring nitrogens, which are 2 – 3 bonds away. No TFA titrated.

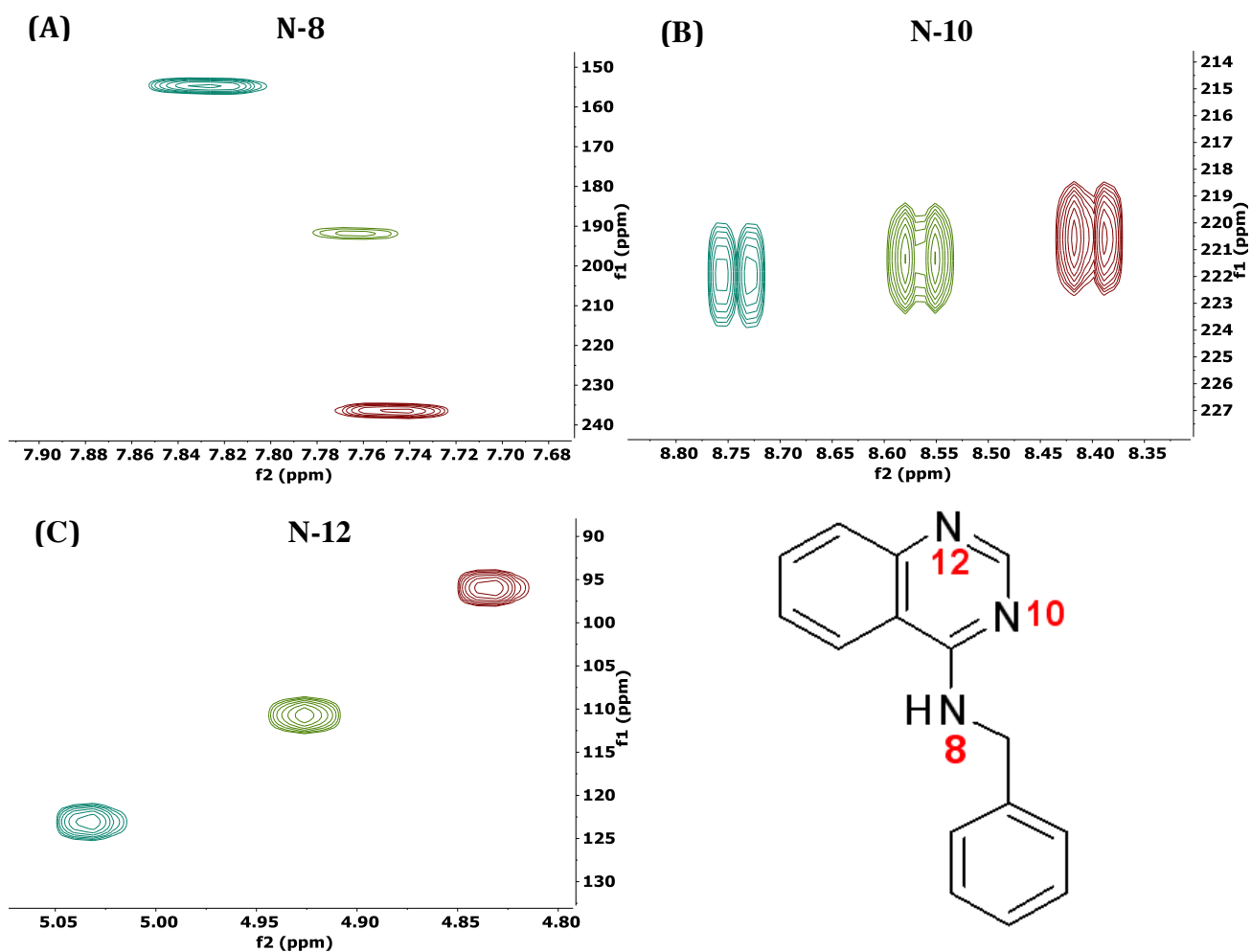


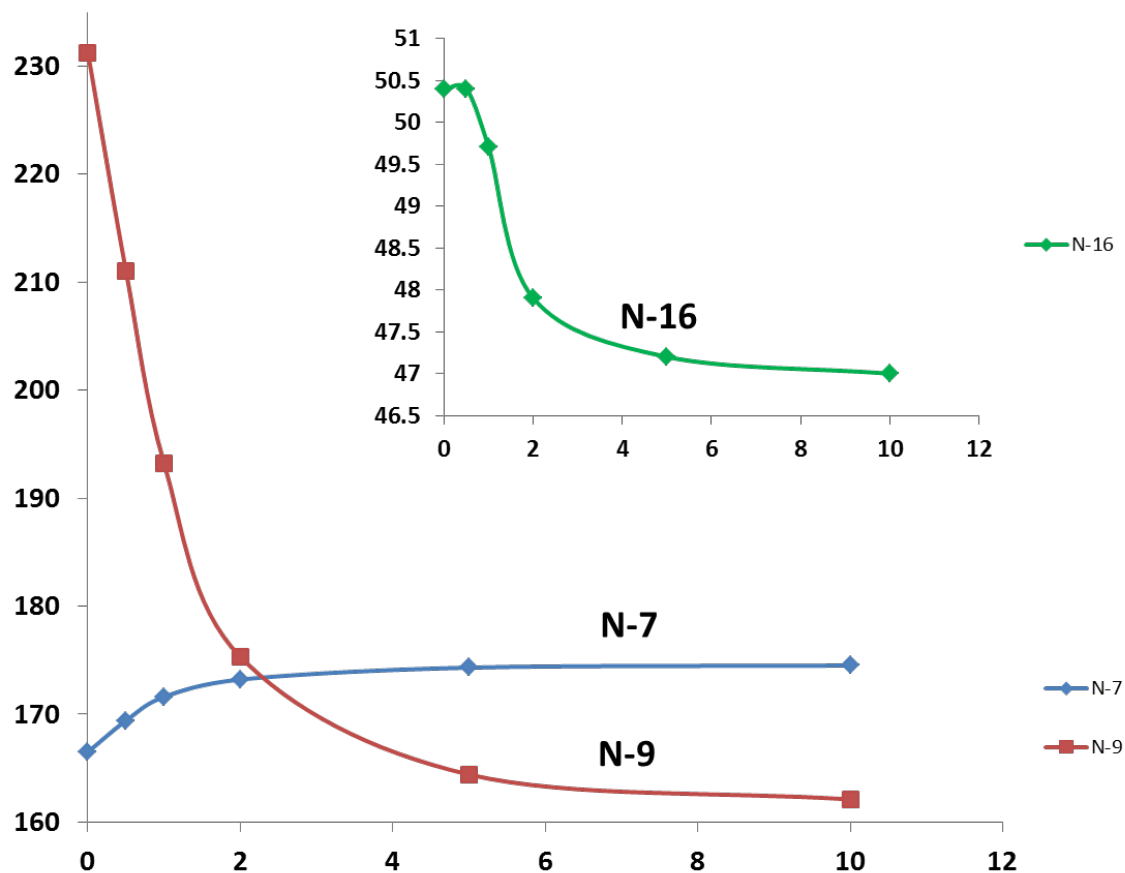


**Figure SI 33.** A 12 scan  $^1\text{H}$ - $^{15}\text{N}$  HMBC spectrum of SM07 (5.8 mg) acquired on a Bruker 500 MHz spectrometer equipped with a 5 mm TCI CryoProbe<sup>TM</sup> prodigy at 298 K in 0.6 ml water- $d_2$ /methanol- $d_4$  (1:2). The  $^{15}\text{N}$  chemical shifts have been assigned by their correlation to near-by protons (no shown). 1.5 equivalents of TFA titrated.



**Figure SI 34.** A 40 scan  $^1\text{H}$ - $^{15}\text{N}$  HMBC spectrum of SM07 (5.8 mg) acquired on a Bruker 500 MHz spectrometer equipped with a 5 mm TCI CryoProbe<sup>TM</sup> prodigy at 298 K in 0.6 ml water- $d_2$ /methanol- $d_4$  (1:2). The  $^{15}\text{N}$  chemical shifts have been assigned by their correlation to near-by protons (not shown). 5 equivalents of TFA titrated.

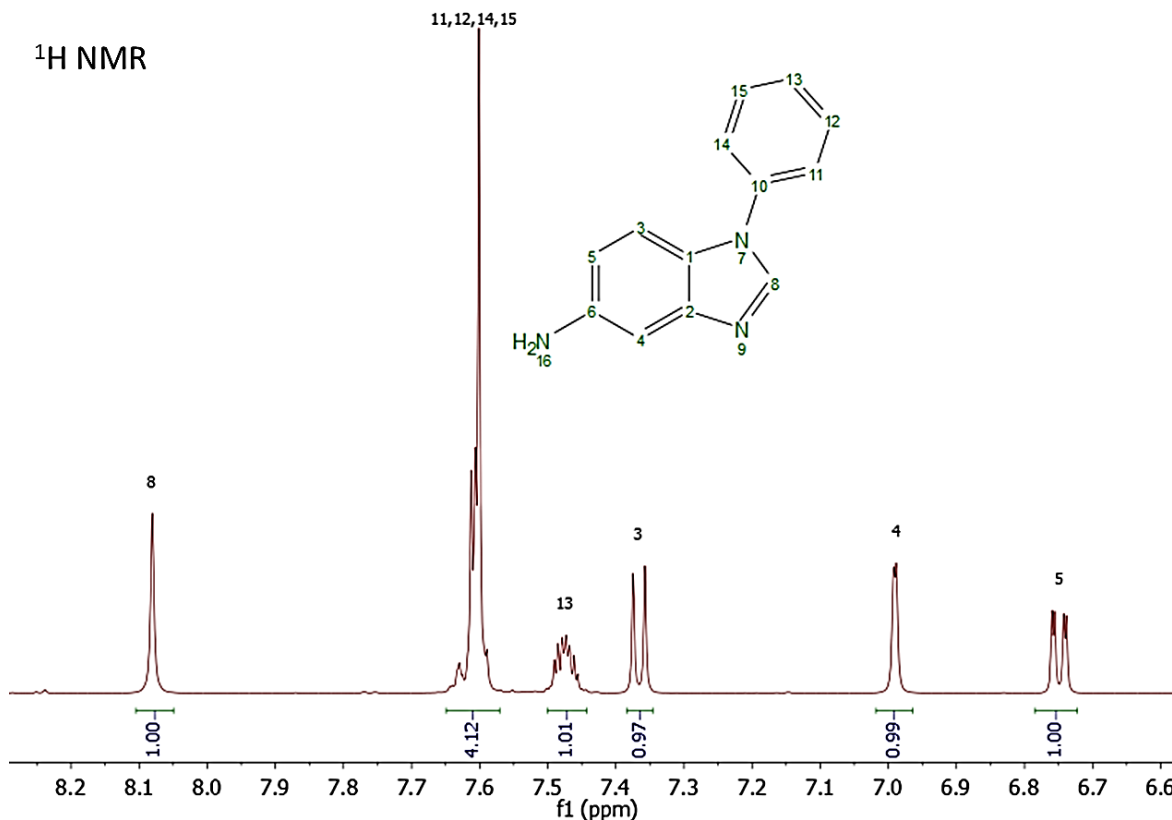




**Figure SI 36.** Plots of NMR observed chemical shifts of N-7, N-9 and N-16 of SM14 vs. TFA-*d* equivalents titrated in water/methanol (1:2), showing strong solvent effects which complicated the data interpretation. For instance, the plots showed continued protonation of N-9 even after 5 equivalents of TFA-*d*. <sup>1</sup>H-<sup>15</sup>N HMBC experiments (2048 x 256 data points) used for the indirect observation of <sup>15</sup>H chemical shifts were acquired on a Bruker 500 MHz spectrometer equipped with a 5 mm TCI CryoProbe<sup>TM</sup> prodigy at 298 K.

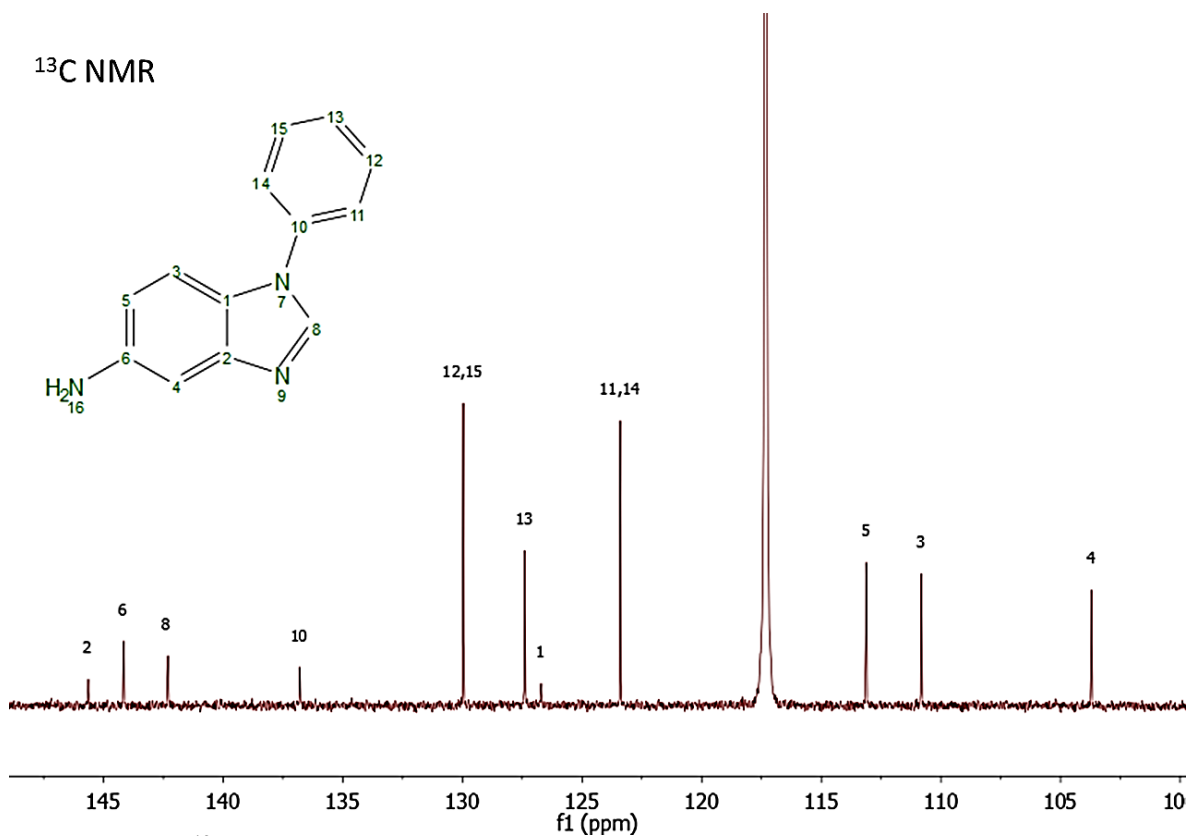


# <sup>1</sup>H NMR

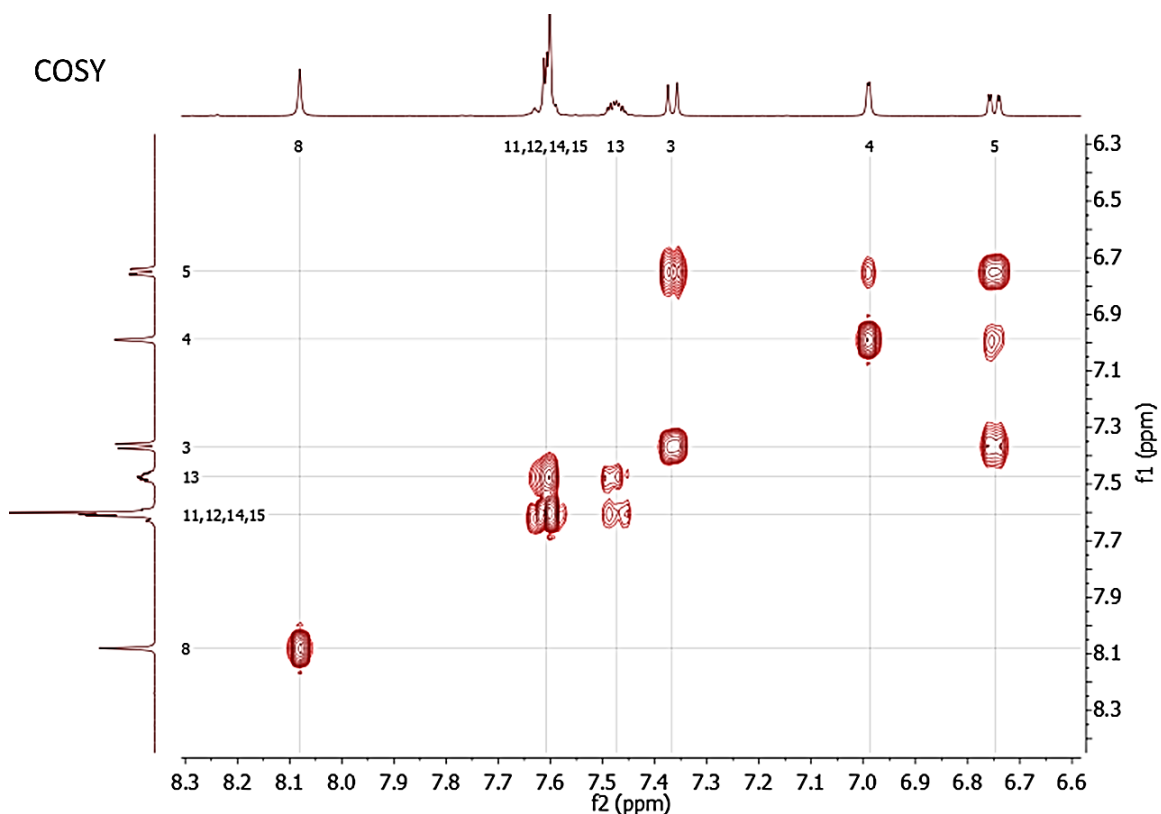


**Figure SI 37.** <sup>1</sup>H NMR spectrum (8 scans) of SM14 (5.5 mg) showing resonances assigned to the corresponding protons. The spectrum was acquired on a Bruker 500 MHz spectrometer equipped with a 5 mm TCI CryoProbe™ prodigy at 298 K in 0.6 ml acetonitrile-*d*<sub>3</sub>.

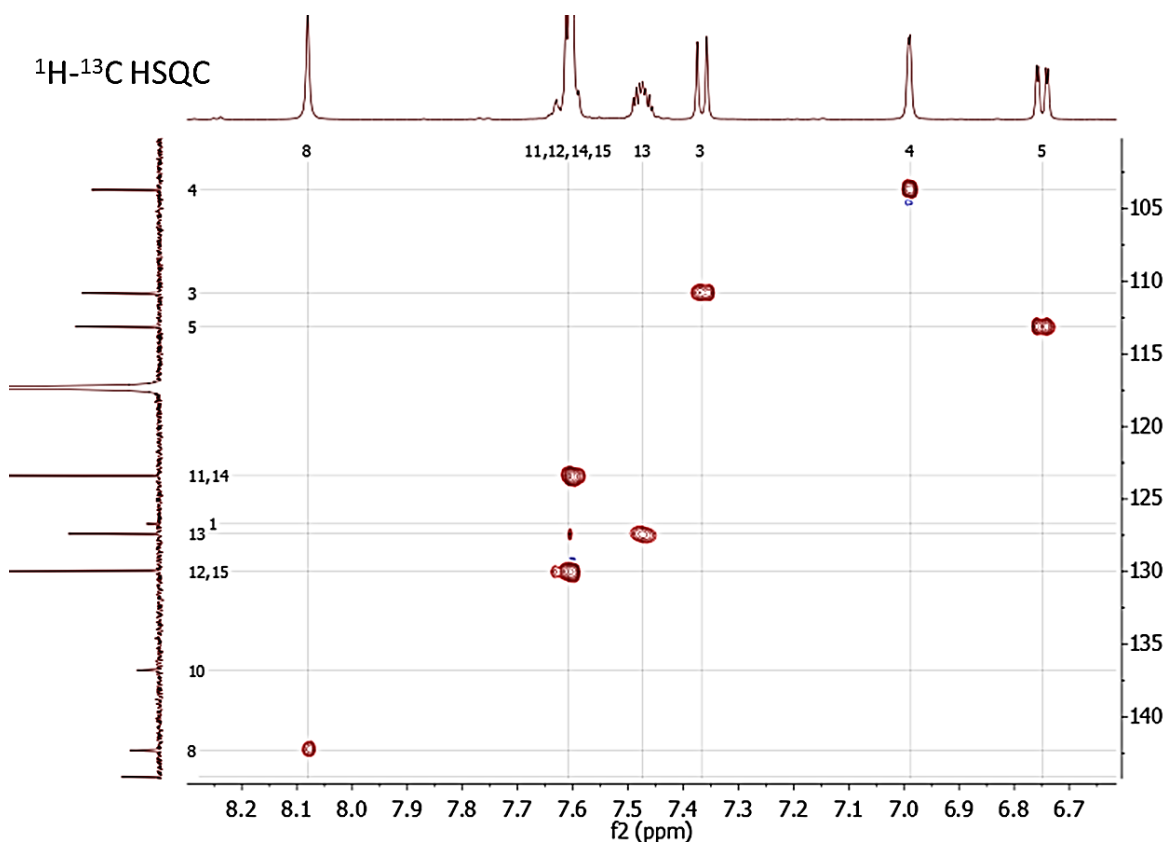
# <sup>13</sup>C NMR



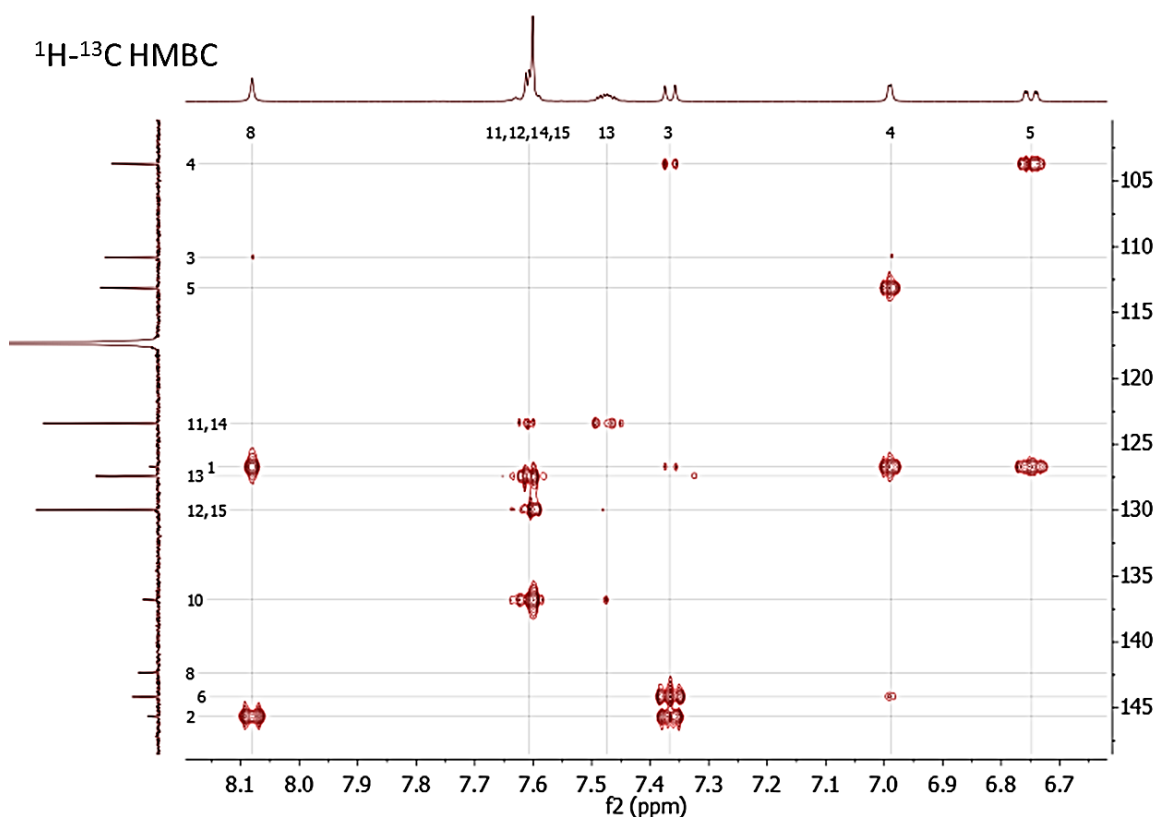
**Figure SI 38.** <sup>13</sup>C NMR spectrum (256 scans) of SM14 (5.5 mg) showing resonances assigned to the corresponding protons. The spectrum was acquired on a Bruker 500 MHz spectrometer equipped with a 5 mm TCI CryoProbe™ prodigy at 298 K in 0.6 ml acetonitrile-*d*<sub>3</sub>.



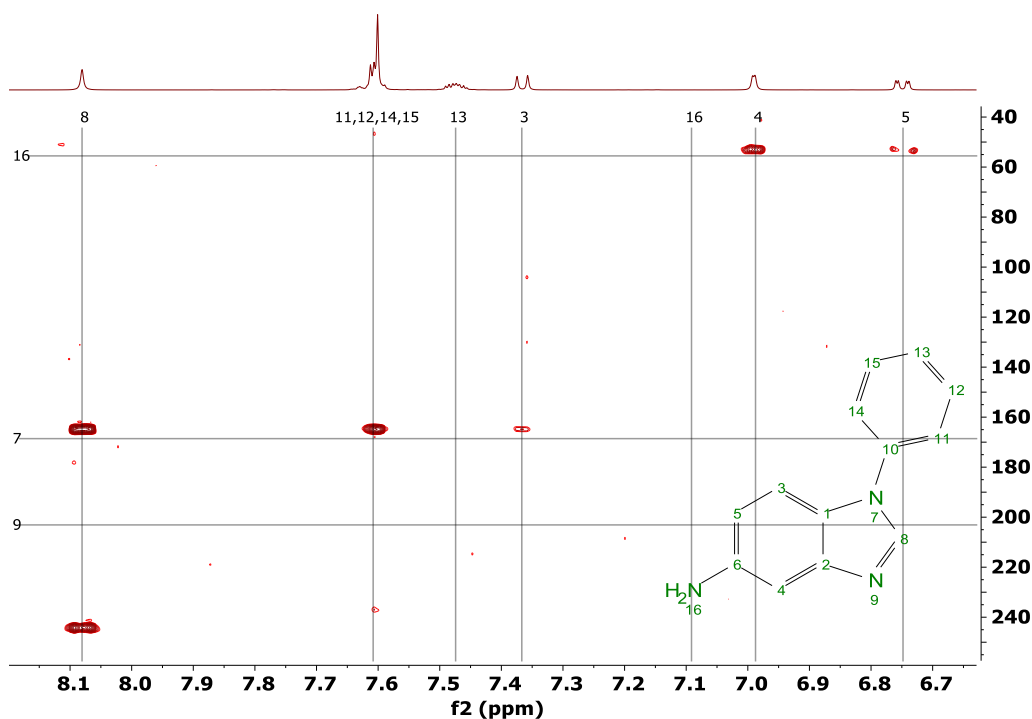
**Figure SI 39.** A 2 scan COSY spectrum of SM14 (5.5mg) acquired on a Bruker 500 MHz spectrometer equipped with a 5 mm TCI CryoProbe™ prodigy at 298 K in 0.6 ml acetonitrile- $d_3$ . Cross-peaks (off-diagonal peaks) corresponds to coupled adjacent protons.



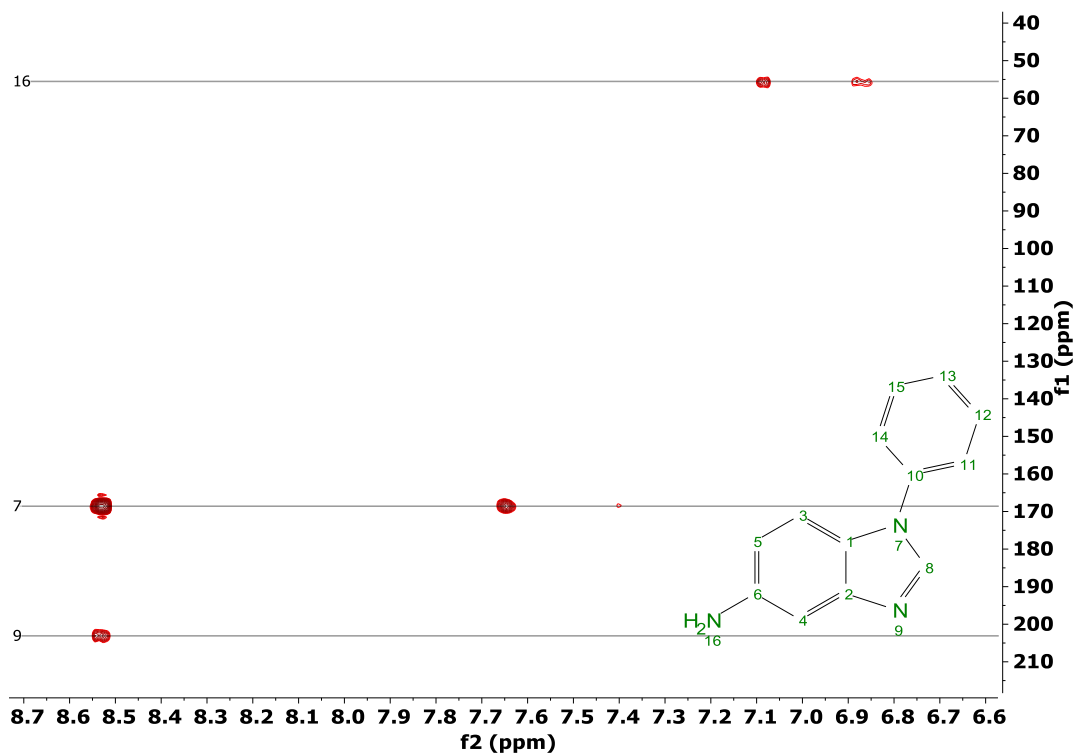
**Figure SI 40.** A 2 scan  $^1\text{H}$ - $^{13}\text{C}$  HSQC spectrum of SM14 (5.5mg) acquired on a Bruker 500 MHz spectrometer equipped with a 5 mm TCI CryoProbe™ prodigy at 298 K in 0.6 ml acetonitrile- $d_3$ . Each peak correlates a carbon to its attached proton(s).



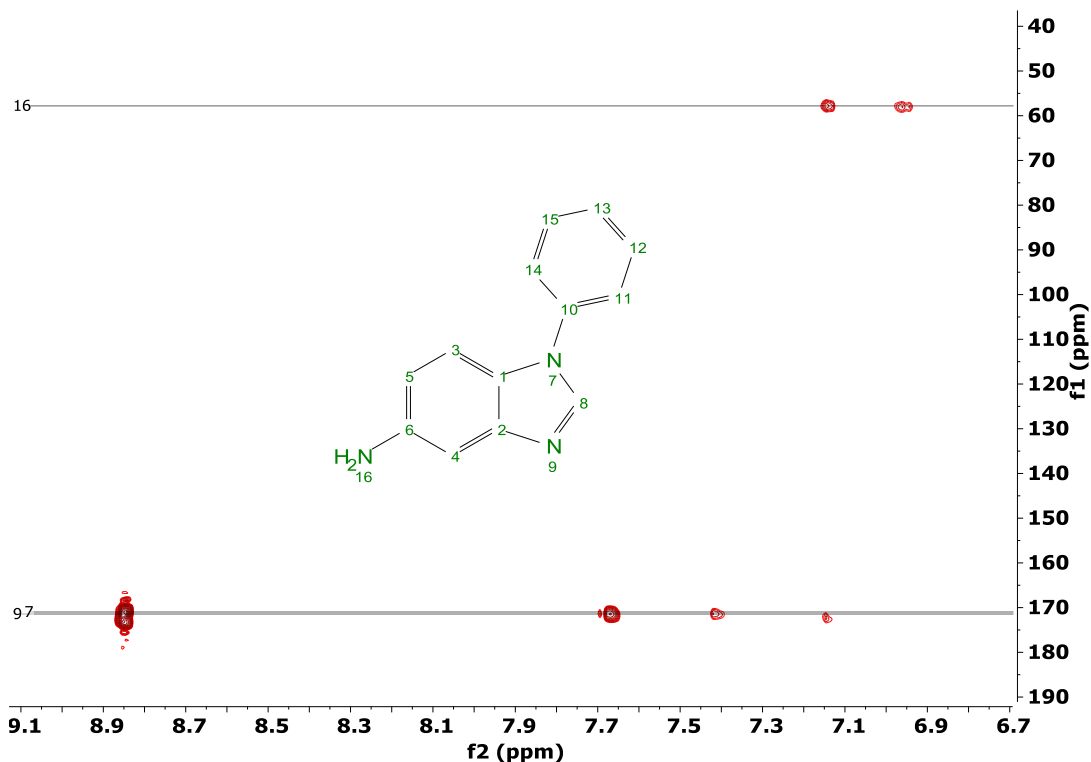
**Figure SI 41.** A 2 scan  $^1\text{H}$ - $^{13}\text{C}$  HMBC spectrum of SM14 (5.5mg) acquired on a Bruker 500 MHz spectrometer equipped with a 5 mm TCI CryoProbe<sup>TM</sup> prodigy at 298 K in 0.6 ml acetonitrile- $d_3$ . Peaks correspond to coupling between the proton of interest to neighboring nitrogens, which are 2 – 3 bonds away.



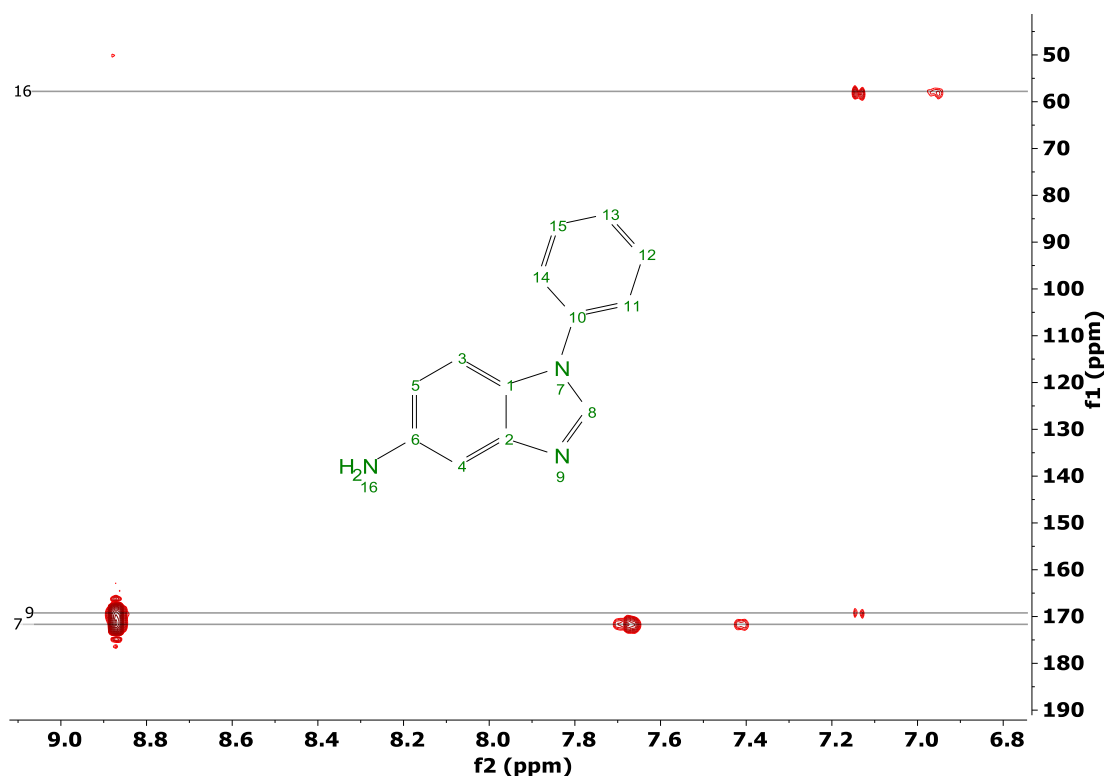
**Figure SI 42.** A 4 scan  $^1\text{H}$ - $^{15}\text{N}$  HMBC spectrum of SM14 (5.5 mg) acquired on a Bruker 500 MHz spectrometer equipped with a 5 mm TCI CryoProbe<sup>TM</sup> prodigy at 298 K in 0.6 ml acetonitrile- $d_3$ . The  $^{15}\text{N}$  chemical shifts have been assigned by their correlation to near-by protons. No TFA titrated.



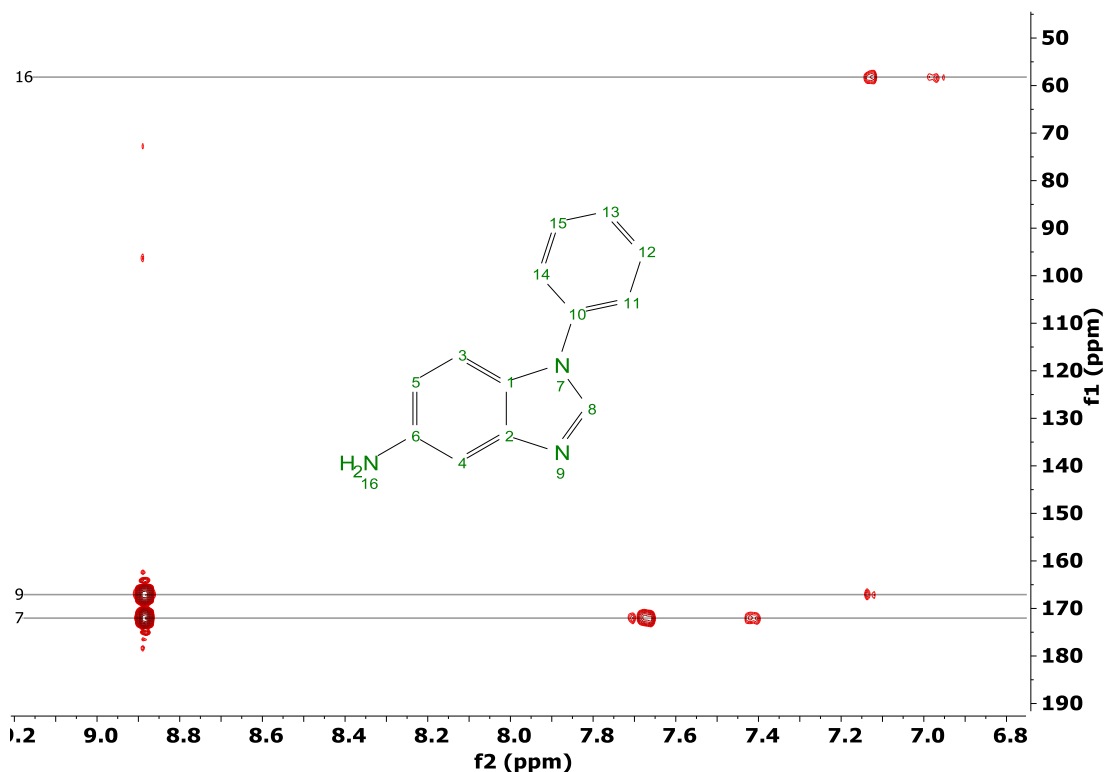
**Figure SI 43.** A 4 scan  $^1\text{H}$ - $^{15}\text{N}$  HMBC spectrum of SM14 (5.5 mg) acquired on a Bruker 500 MHz spectrometer equipped with a 5 mm TCI CryoProbe<sup>TM</sup> prodigy at 298 K in 0.6 ml acetonitrile- $d_3$  after titration with 0.5 equivalents of TFA. The  $^{15}\text{N}$  chemical shifts have been assigned by their correlation to near-by protons (not shown).



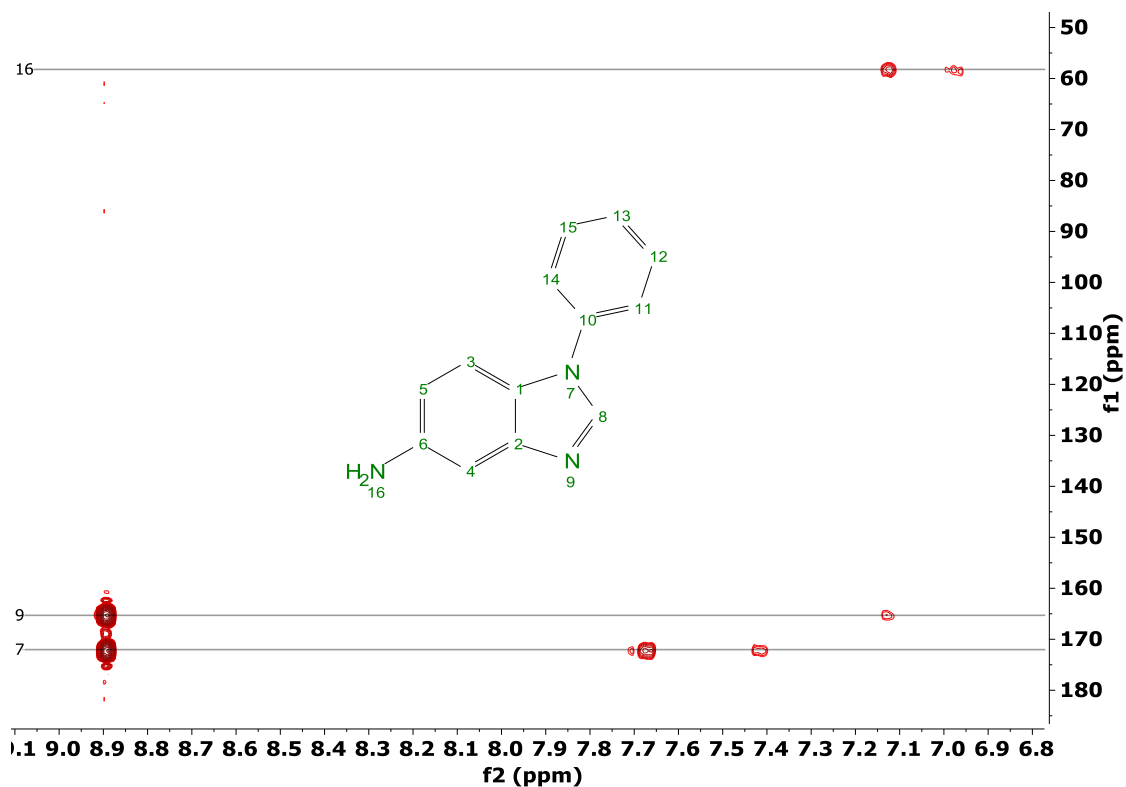
**Figure SI 44.** A 4 scan  $^1\text{H}$ - $^{15}\text{N}$  HMBC spectrum of SM14 (5.5 mg) acquired on a Bruker 500 MHz spectrometer equipped with a 5 mm TCI CryoProbe<sup>TM</sup> prodigy at 298 K in 0.6 ml acetonitrile- $d_3$  after titration with 1.0 equivalents of TFA. The  $^{15}\text{N}$  chemical shifts have been assigned by their correlation to near-by protons (not shown).



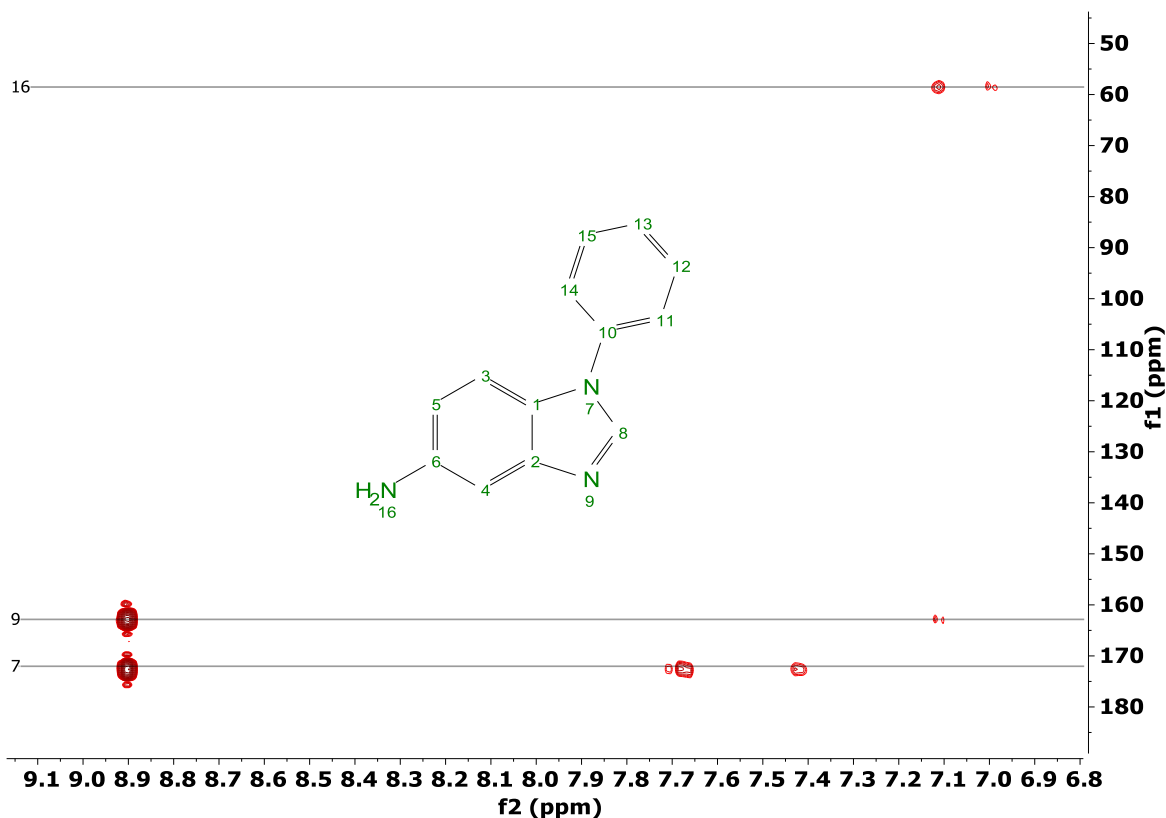
**Figure SI 45.** A 4 scan  $^1\text{H}$ - $^{15}\text{N}$  HMBC spectrum of SM14 (5.5 mg) acquired on a Bruker 500 MHz spectrometer equipped with a 5 mm TCI CryoProbe<sup>TM</sup> prodigy at 298 K in 0.6 ml acetonitrile- $d_3$  after titration with 1.1 equivalents of TFA. The  $^{15}\text{N}$  chemical shifts have been assigned by their correlation to near-by protons (not shown).



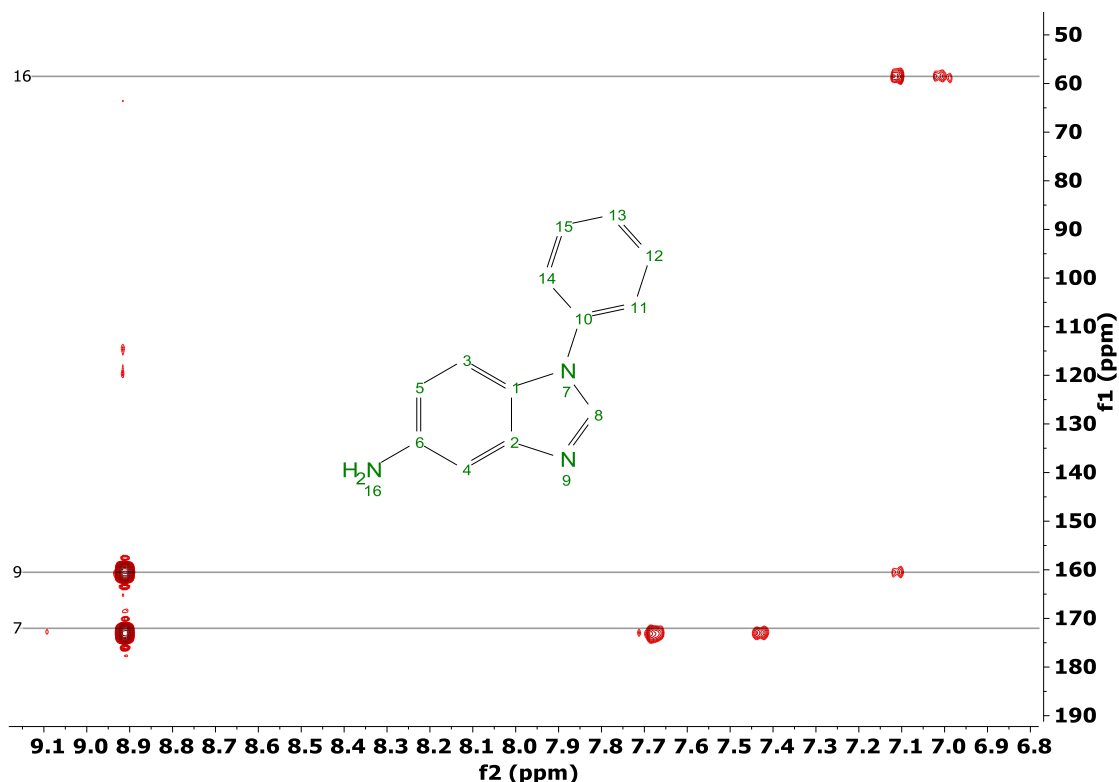
**Figure SI 46.** A 4 scan  $^1\text{H}$ - $^{15}\text{N}$  HMBC spectrum of SM14 (5.5 mg) acquired on a Bruker 500 MHz spectrometer equipped with a 5 mm TCI CryoProbe<sup>TM</sup> prodigy at 298 K in 0.6 ml acetonitrile- $d_3$  after titration with 1.2 equivalents of TFA. The  $^{15}\text{N}$  chemical shifts have been assigned by their correlation to near-by protons (not shown).



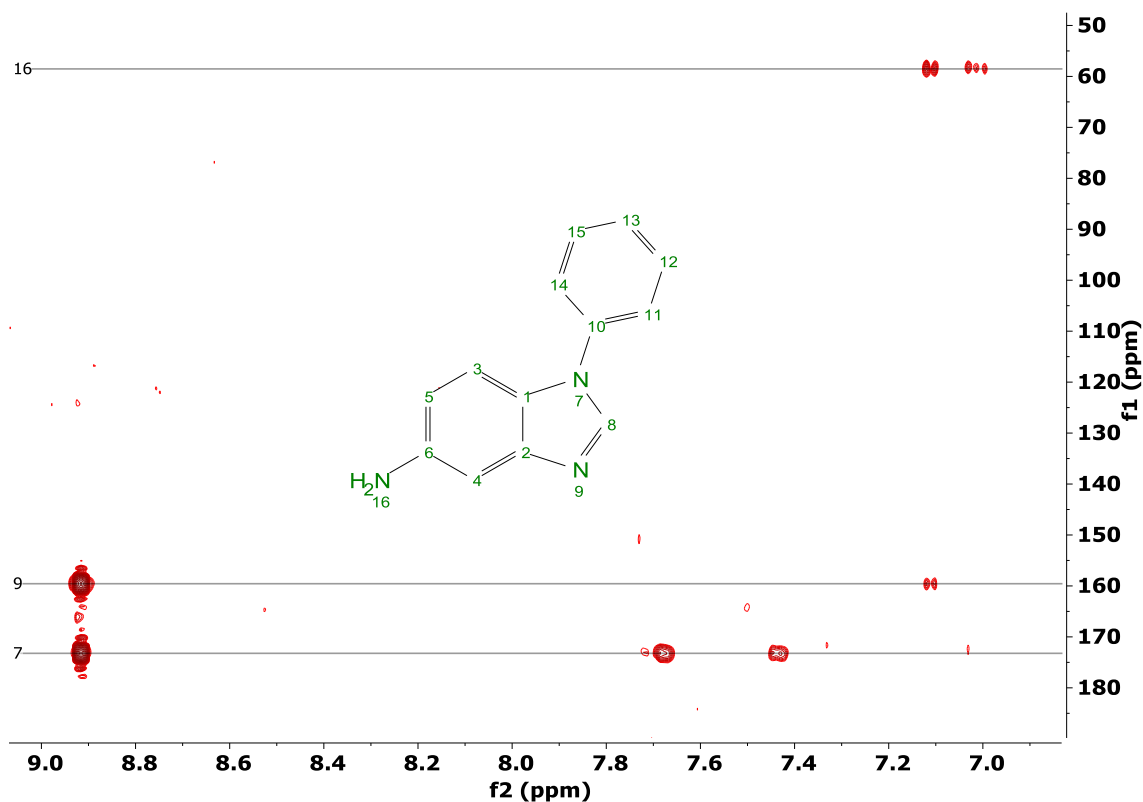
**Figure SI 47.** A 4 scan  $^1\text{H}$ - $^{15}\text{N}$  HMBC spectrum of SM14 (5.5 mg) acquired on a Bruker 500 MHz spectrometer equipped with a 5 mm TCI CryoProbe<sup>TM</sup> prodigy at 298 K in 0.6 ml acetonitrile- $d_3$  after titration with 1.3 equivalents of TFA. The  $^{15}\text{N}$  chemical shifts have been assigned by their correlation to near-by protons (not shown).



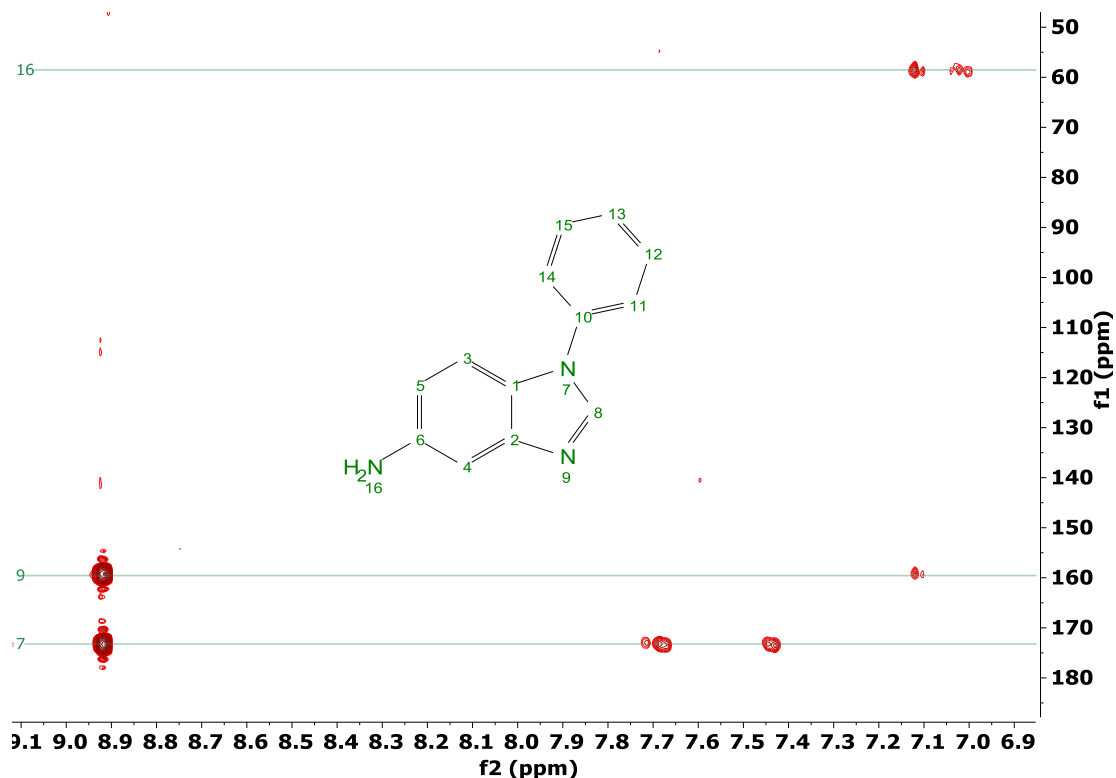
**Figure SI 48.** A 4 scan  $^1\text{H}$ - $^{15}\text{N}$  HMBC spectrum of SM14 (5.5 mg) acquired on a Bruker 500 MHz spectrometer equipped with a 5 mm TCI CryoProbe<sup>TM</sup> prodigy at 298 K in 0.6 ml acetonitrile- $d_3$  after titration with 1.5 equivalents of TFA. The  $^{15}\text{N}$  chemical shifts have been assigned by their correlation to near-by protons (not shown).



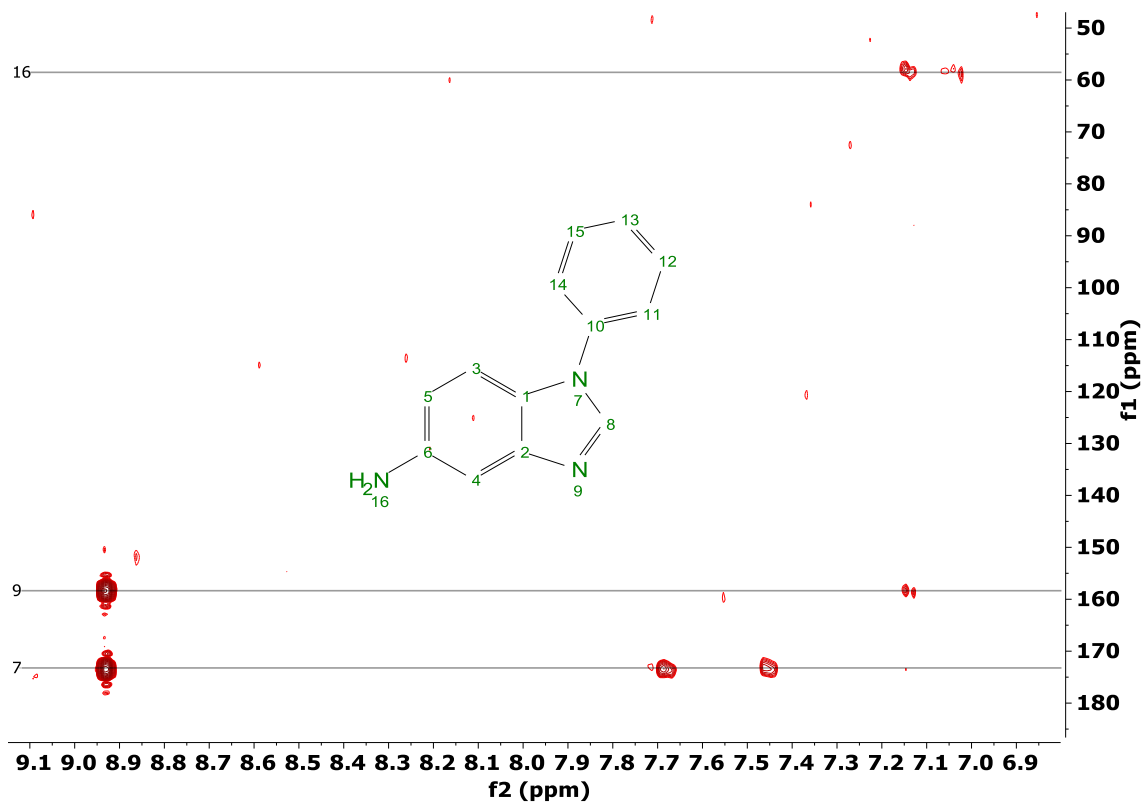
**Figure SI 49.** A 4 scan  $^1\text{H}$ - $^{15}\text{N}$  HMBC spectrum of SM14 (5.5 mg) acquired on a Bruker 500 MHz spectrometer equipped with a 5 mm TCI CryoProbe<sup>TM</sup> prodigy at 298 K in 0.6 ml acetonitrile- $d_3$  after titration with 1.8 equivalents of TFA. The  $^{15}\text{N}$  chemical shifts have been assigned by their correlation to near-by protons (not shown).



**Figure SI 50.** A 4 scan  $^1\text{H}$ - $^{15}\text{N}$  HMBC spectrum of SM14 (5.5 mg) acquired on a Bruker 500 MHz spectrometer equipped with a 5 mm TCI CryoProbe<sup>TM</sup> prodigy at 298 K in 0.6 ml acetonitrile- $d_3$  after titration with 2.0 equivalents of TFA. The  $^{15}\text{N}$  chemical shifts have been assigned by their correlation to near-by protons (not shown).

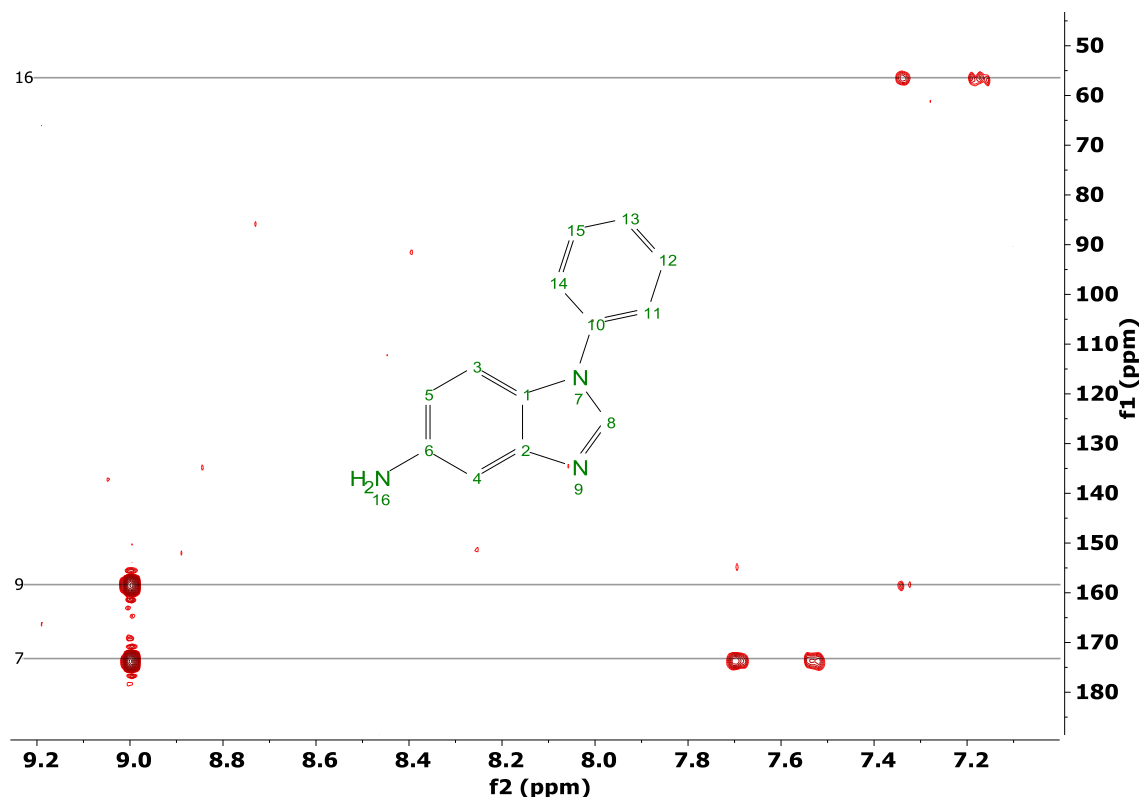


**Figure SI 51.** A 4 scan  $^1\text{H}$ - $^{15}\text{N}$  HMBC spectrum of SM14 (5.5 mg) acquired on a Bruker 500 MHz spectrometer equipped with a 5 mm TCI CryoProbe<sup>TM</sup> prodigy at 298 K in 0.6 ml acetonitrile- $d_3$  after titration with 2.1 equivalents of TFA. The  $^{15}\text{N}$  chemical shifts have been assigned by their correlation to near-by protons (not shown).

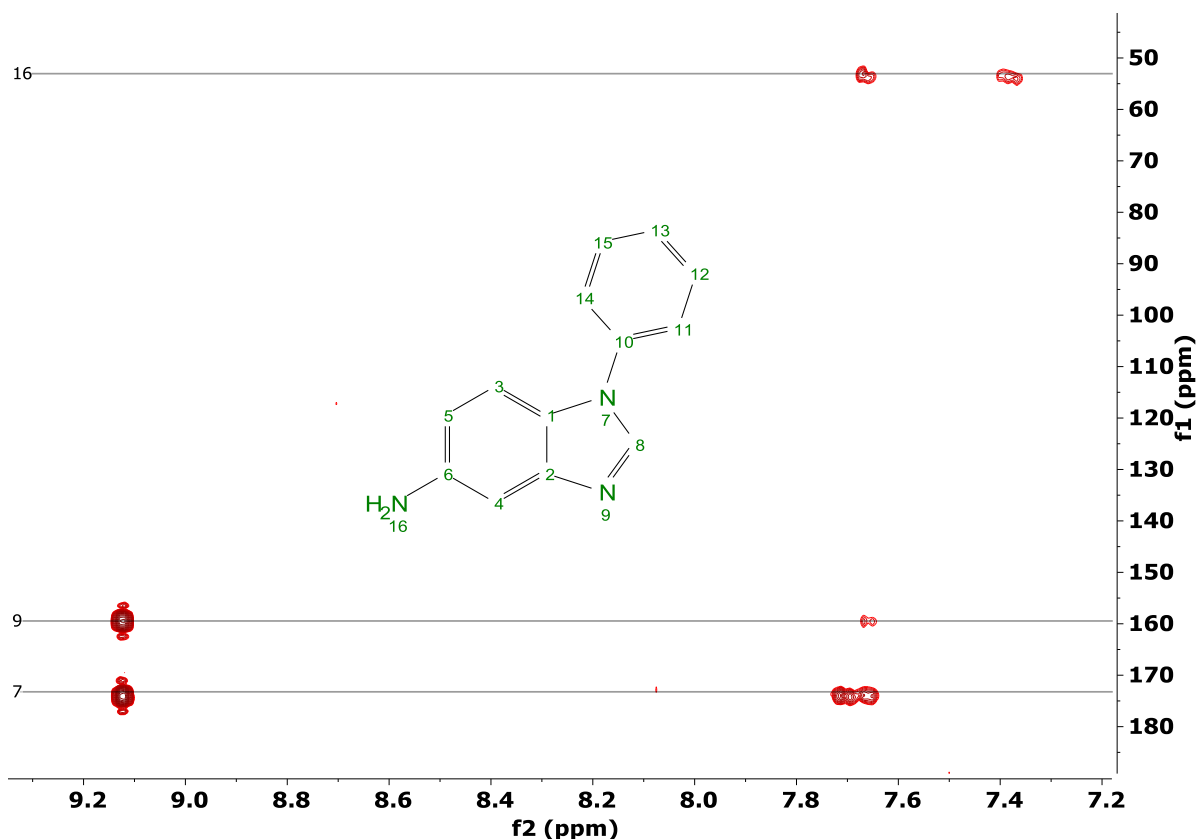


**Figure SI 52.** A 4 scan  $^1\text{H}$ - $^{15}\text{N}$  HMBC spectrum of SM14 (5.5 mg) acquired on a Bruker 500 MHz spectrometer equipped with a 5 mm TCI CryoProbe<sup>TM</sup> prodigy at 298 K in 0.6 ml acetonitrile- $d_3$  after titration with 2.6 equivalents of TFA. The  $^{15}\text{N}$  chemical shifts have been assigned by their correlation to near-by protons (not shown).





**Figure SI 53.** A 4 scan  $^1\text{H}$ - $^{15}\text{N}$  HMBC spectrum of SM14 (5.5 mg) acquired on a Bruker 500 MHz spectrometer equipped with a 5 mm TCI CryoProbe<sup>TM</sup> prodigy at 298 K in 0.6 ml acetonitrile- $d_3$  after titration with 5.1 equivalents of TFA. The  $^{15}\text{N}$  chemical shifts have been assigned by their correlation to near-by protons (not shown).



**Figure SI 54.** An 8 scan  $^1\text{H}$ - $^{15}\text{N}$  HMBC spectrum of SM14 (5.5 mg) acquired on a Bruker 500 MHz spectrometer equipped with a 5 mm TCI CryoProbe<sup>TM</sup> prodigy at 298 K in 0.6 ml acetonitrile- $d_3$  after titration with 10.1 equivalents of TFA. The  $^{15}\text{N}$  chemical shifts have been assigned by their correlation to near-by protons (not shown).